F.

22200180002

Med
K28337

Links in a Chain of Research on Syphilis (Oxidation and Reduction).

Being the Hunterian Lectures delivered before
the Royal College of Surgeons, March, 1916,

BY

J. E. R. McDONAGH, F.R.C.S.

LONDON
HARRISON AND SONS, 45 PALL MALL, S.W.
1916.

94132

13356

Coll. welMOmec
Call
No. WC

CONTENTS.

CHAPTER I.

PAGE

History (1786—1916) 1

CHAPTER II.

The Life-Cycle of the Organism of Syphilis (*Leucocytozoon syphilidis*) 7

CHAPTER III.

Dyeing and Staining 12

CHAPTER IV.

A Sketch of the Main Theories of Immunity up to the Discovery of the Wassermann Reaction, with Special Reference to the Nature of Complement 24

CHAPTER V.

The Wassermann Reaction 31

CHAPTER VI.

Immunity and Colloids 40

CHAPTER VII.

A New and Rational Test for Syphilis (the Emulsoid-Suspensoid, or the E-S. Test) 75

CHAPTER VIII.

Anaphylaxis 91

CHAPTER IX.

Chemotherapy 104

CHAPTER X.

The Significance of the Hydrogen and Hydroxyl Ions in Biology 165

SUMMARY 187

INDEX 201

CHAPTER I.

HISTORY (1786–1916).

BEFORE dealing with the main theme of these lectures, it would be as well to glance over the advances made in our knowledge of syphilis, since the eighteenth century, as, by so doing, it will be easy to show how we have arrived at our present state of knowledge.

I propose to take as my first milestone, the work done by John Hunter[1] in the latter part of the eighteenth century.

It will be remembered that, about that time, the burning question of the day was whether gonorrhœa and syphilis were forms or stages of one and the same disease, or whether they were two different diseases.

To settle the question, Hunter inoculated himself with the discharge from a case of gonorrhœa, and developed both gonorrhœa and syphilis, with the result that the unicity of the two diseases appeared to be established.

Although many observers were still sceptical, and really believed in the duality of the diseases, it was not until about a half-a-century later that Ricord[2] finally proved to the world that the dualists were correct.

Hunter's opinion would never have held sway for the long time it did, except for the extraordinary respect which all seemed to have for him and for his work.

There is no doubt that Ricord allowed his better judgment, in the earlier part of his career, to be outbalanced by the reverence in which Hunter's name was held; but, having once proved that gonorrhœa and syphilis were distinct diseases, he was easily able to show that Hunter's other clinical remarks about syphilis were incorrect—namely, that syphilis never attacked the brain, heart, stomach, liver, kidneys and intestines. It is strange that a man like Hunter should have made such a bad clinical mistake, since several reports of visceral syphilis had appeared before Hunter's time.

Ricord, a Frenchman, was undoubtedly the first to give us a clear account of the clinical features of syphilis, and most of his work stands as good to-day as when he wrote it.

Although Ricord distinguished clinically between a hard and a soft sore, he thought, almost up to the end of his life, that both kinds of sore represented the same disease, namely syphilis. Bassereau,[3] one of Ricord's pupils, showed clinically that the two sores had a different ætiology, and his opinion was still further supported by Rollet[4] in Lyon, Waller[5] in Prague, v. Zeissl[6] and Hebra[7] in Vienna, and v. Baerensprung[8] in Berlin.

Owing to the evidence brought forward in favour of the dual infection, Ricord at last gave up his unitary view, and in so doing said, "*L'homme absurde est qui ne change pas.*"

Naturally, the fact that a soft sore (*Ulcus molle*) was a distinct infection, could not be scientifically proved until Ducrey[9] had found the causative organism in 1889.

Our clinical knowledge of syphilis was greatly added to after Ricord's time by another Frenchman, Fournier[10], and by an Englishman, Jonathan Hutchinson[11].

Both these men held the palm for increasing our knowledge of syphilis, until it left us for Germany in the year 1905. From 1905 onwards, up to the present time, we have been served up with so-called epoch-making discoveries by Germans, and no people have more devoutly worshipped them than the English. In 1905, Schaudinn and Hoffmann[12] discovered the *Spirochæta pallida*, which is, even up to the present time, generally regarded as the cause of syphilis.

In 1907 the Wassermann[13] reaction saw daylight, and in 1909 we had Ehrlich's[14] discovery of salvarsan.

Let us return to the year 1905, and let us see how the discovery of the *Spirochæta pallida* was made.

The opinion was held that the probable cause of syphilis was a protozoon, hence it was suggested to Schaudinn, who was one of the leading protozoologists of the day, that he should examine some syphilitic material for protozoa.

Schaudinn, as he lived in Bonn, obtained his material from Hoffmann, who had charge of the Venereal Department of the University at that time.

I think I am right in saying that the *Spirochæta pallida* was found in the first material examined, and the discovery was confirmed by the subsequent examinations. The world was then told that the cause of syphilis had been discovered. Everyone hastened to repeat the work, and in a very short time the *Spirochæta pallida* had been found in almost every known syphilitic lesion. Although one or two observers tried to ascertain what kind of an organism the *Spirochæta pallida* was, most were perfectly content to regard it as an organism which had no other phase, in spite of the fact that no one had succeeded in actually witnessing division—surely a peculiar occurrence, considering that no more perfect methods of demonstrating an organism existed, and that no organism was more frequently searched for and studied.

I shall leave the *Spirochæta pallida* for a moment, and turn to the Wassermann reaction.

Pathologists, in the years just prior to 1907, had been paying considerable attention to the problems of immunity, with the result that various methods had been discovered for the detection of specific bacterial infections. One of the most important of these was Bordet and Gengou's[15] complement-fixation test, by means of which an infection could be diagnosed by finding its antibody in the sernm.

In order to carry out the complement-fixation test, it was necessary to have an extract of bacteria, the antibody of which was to be sought. As the *Spirochæta pallida* had, up to this time, resisted all attempts to be cultured, Wassermann made an extract of syphilitic fœtal liver, an organ which had been proved to be especially rich in specific spirochaetæ, and he used this in place of an extract of the cultured organism.

The modified complement-fixation test was found to be positive in cases of syphilis. Like the discovery of the

Spirochæta pallida, the Wassermann reaction very soon became general, and we all rushed to the conclusion that a positive reaction always meant that a patient had active syphilis, and therefore required treatment, and that a negative reaction often meant that the patient was cured, in spite of two glaring facts: (1) that an extract of non-spirochætal tissue would serve equally well as the antigen; (2) that the *rationale* of the reaction was a complete enigma.

Two years later, *i.e.*, in 1909, Ehrlich discovered salvarsan. As the *rationale* of chemotherapy is to form the main theme of my lectures, I will not, for the moment, go more closely into Ehrlich's discovery, except to mention one point, upon which hangs what I must deal with first. It was a recognised fact that, 24 hours after the first injection of salvarsan, no *Spirochætæ pallidæ* could be found in the syphilitic lesions, yet it was necessary to repeat the injections of salvarsan several times, and to prescribe mercury afterwards, to avoid recurrences taking place. In many cases, in spite of following out the above, recurrences did take place, as they were wont to do when mercury only was employed. This paradox led me to doubt whether the statements made about the German discoveries were correct, and, looking at the whole subject, as I did in 1911, with a very open mind, three pertinent questions confronted me, a solution to which I could not find with the knowledge as it then existed. The three questions were the following:—

(1) If the *Spirochæta pallida* is the cause of syphilis, why do we never see it divide?

(2) If all the spirochætæ are killed within 24 hours after the first injection of "606," why is it necessary to continue treatment?

(3) If the *Spirochæta pallida* cannot pass through the placenta, how can an apparently healthy woman bear an actively syphilitic child, since it is impossible to imagine that an organism, the length of a *Spirochæta pallida*, should enter the ovum with a spermatozoon.

It then occurred to me that the *Spirochæta pallida* was not the absolute cause of syphilis, but possibly only a phase in the life-history of a protozoon.

Owing to the fact that the *Spirochæta pallida* did not appear to multiply by division, or to alter in any way at all, and owing to its close resemblance to male gametes of other protozoa, and even to the spermatozoon itself, it struck me that the organism might perhaps be the adult male or, at any rate, an end phase of a complicated protozoal life-history.

In 1911[16] I began to examine lymphatic glands from the region draining the site of the initial lesion. These glands had been removed early in the generalisation stage. I chose lymphatic glands and not chancres at first, so as to avoid being misled by extraneous organisms and broken-down cells, both of which occur in abundance in erosive and ulcerative lesions. I soon found some bodies which I thought might be parasitic in nature, as they had special staining properties, and could not be demonstrated in the control material.

As a result of further investigation, with fresh and fixed tissue, I[17] was able to work out the life-cycle of what was evidently a coccidial protozoon.

As a result of some chemical investigations, I found that the phases of the protozoon consisted of a protein, which had special properties.

This, then, gave me a clue which resulted in my being able to explain the *rationale* or *modus operandi* of the Wassermann reaction.[18]

The Wassermann reaction turned out to be a physical reaction, dependent upon certain properties which the protein particles of syphilitic sera naturally possessed.

Having found out what these properties were, it became clear how salvarsan worked.

Further investigation into this subject soon unravelled the *rationale* of chemotherapy, and opened the way along which future research might be expected to be the most productive.

The first drug I[19] prepared turned out to be better in many respects than any anti-syphilitic remedy which had previously been discovered, showing that each piece of work dove-tailed into the other, the best proof of their correctness. I will attempt to traverse the ground again, and to show how each step follows on the other ; therefore I will begin by mentioning a few words about the life-cycle of the organism of syphilis.

REFERENCES.

[1] Hunter (1786), "Treatise on the Venereal Disease." London.

[2] Ricord (1863), "Lettres sur la Syphilis." Paris.

[3] Bassereau (1852), "Traité des affections de la peau symptomatiques de la Syphilis," Baillière, Paris.

[4] Rollet (1861–62), "Recherches cliniques et expérimentales sur la Syphilis." Ballière, Paris.

[5] Waller (1859–60), "Vierteljähr. f. d. prakt. Heilkunde," Prag, lxiii, 135 ; *ibid.*, lxvi, 133.

[6] v. Zeissl (1864), "Lehrbuch der constitutionellen Syphilis." Enke, Erlangen.

[7] v. Baerensprung (1853–55), "Deutsche Klinik," v, 511 ; *ibid.*, vi, 508 ; *ibid.*, vii, 34.

[8] v. Baerensprung (1861), "Deutsche Klinik," xiii, 30.

[9] Ducrey (1889), "Giorn. ital. d. mal. Ven. e. d. Pelle," xxx, 377.

[10] Fournier (1906), "Traité de la Syphilis," 2 vols. Rueff, Paris.

[11] J. Hutchinson (1901), "Syphilis." Cassell & Co., London.

[12] Schaudinn u. Hoffmann (1905), "Arb. a. d. Kais. Gesundheitsamte," xxii, 527.

[13] Wassermann u. Bruck (1906), "Deutsch. med. Woch.," xxxii, 450.

[14] Ehrlich u. Hata (1910), "Die experimentelle Chemotherapie der Spirillosen." Springer, Berlin.

[15] Bordet et Gengou (1901), "Ann. de l'Instit. Pasteur," xv, 289.

[16] McDonagh (1915), "The Biology and Treatment of Venereal Diseases." Harrison & Sons, London.

[17] McDonagh (1914), "Arch. f. Derm. u. Syph.," cxix, 205.

[18] McDonagh (1915), "Quart. Journ. of Medicine," viii, 129.

[19] McDonagh (1916), "Lancet," i, 236.

CHAPTER II.

THE LIFE-CYCLE OF THE ORGANISM OF SYPHILIS (*LEUCOCYTOZOON SYPHILIDIS*).

THE life-cycle commences with a spore, or, as it is generally called, a sporozoite. The sporozoite, when examined *in vivo*, remains for some time unstained. Later, it stains very deeply, but its motility is not thereby impaired. It is about 1·5 microns in diameter, and it is actively motile. Besides being found in the scrapings from syphilitic lesions, it may be found in the blood withdrawn from the healthy skin surrounding a chancre, and also in the general blood-stream, during the stage of general infection. The sporozoite then becomes intracellular. It chooses a connective-tissue cell or an endothelial cell as its host, and, when inside, it undergoes important changes, which can best be described under two headings:—

(1) The sporozoite steadily increases in size, and, by a process of budding, gives rise to several bodies, which later become differentiated into male and female elements. By this time, the cell is a sac, as all the reserve material has been used up by the merozoites; the nucleus still remains, although degenerated, but finally it disappears, when the sac gives way and frees the male and female merozoites. Not all the bodies formed in this way are sexually differentiated; there are others which become free with the sexual merozoites, and are able, no doubt to start the cycle again, by seeking fresh connective-tissue cells or endothelial cells.

(2) The sporozoite increases in size, but not to the dimensions met with in the previous case. Having reached a certain size, it divides into two, and again into four. These four masses, by a process of further subdivision, form a ring, and migrate to the periphery of the body, when a picture is given as if the ring

had stones mounted in it, the whole way round. By this time, the host cell is almost completely degenerated, and one might imagine that the parasite had become extracellular, but it does so only when the host cell is no more. In the centre, and around the ring, other deeply stained bodies appear, until a picture of a perfect spore cyst is given. This is doubtless the true asexual stage, and the two stages just described represent the schizogony.

Both the male and female gametocytes are motile, but not flagellated. The male consists of three nuclear bodies, while the female contains a nucleus at her upper end, and one or two very actively motile blepharoplasts at her lower end. When the female has reached the size of a red blood corpuscle, she loses her blepharoplasts and becomes stationary. Before fertilisation, the nucleus of the female gamete moves from the upper pole, takes a central position, and fills up practically the whole cell.

The male gametocyte may develop intracellularly or extracellularly. If the former, it enters a large mononuclear leucocyte, wherein the three nuclear bodies increase in size, develop into a coil, and from each nuclear body a number of spirochætæ arise, like the spokes of a wheel from its axle. The spirochætæ break loose, and each can then be recognised as a true *Spirochæta pallida.* In the extracellular development, each nuclear body divides and subdivides, so that a rosette-like appearance is formed. Several bodies may break away *en masse,* in the shape of a chain, which ultimately breaks up into distinct coccus-like forms; or discrete coccus-like forms may at first break away. Each coccus-like body contains two rods, which give it the appearance of a diplococcus, and these rods develop into thick and unevenly coiled spirochætæ, which eventually become typical *Spirochætæ pallidæ.*

The immature spirochæta, which is first formed in this extracellular route, resembles the refringens type; therefore it is very probable that a spirochæta, in two stages of its development, is indistinguishable from the *Spirochæta refringens* and the *Spirochæta pallida.*

The act of fertilisation occurs as follows. In some cases, the spirochæta appears to enter the female nucleus, wherein it becomes lost; while, in other cases, it seems to become connected with the female nucleus by a skein. In both cases, the nucleus, which contains both male and female elements, migrates again to the upper pole; such a cell is a zygote.

In the nine instances in which I have witnessed fertilisation, only one spirochæta was seen to enter the female. The spirochæta takes about an hour to become entirely lost to view. While entering, the whole cell is in active motion, but once the spirochæta has entered, the cell comes to a sudden standstill, and appears to become covered with a mantle.

A few minutes after impregnation, a polar body is expelled with considerable force from the cell, and again another, after an interval of a minute or two. During the extrusion of the polar bodies, the cell is very actively motile, but it becomes stationary immediately after the second has been ejected.

The nucleus of the zygote divides and subdivides into sporoblasts; the sporoblasts may further divide and subdivide *in situ,* to form sporozoites; or, a sporoblast may escape and form sporozoites independently.

From the above description of the life-history of the organism of syphilis, I think I am justified in assigning it to the order *Sporozoa* and to the sub-class *Telosporidia,* since the spores are formed at the end of a cycle. The order is doubtless the *Coccidiidea* and the species which most befits it is the *Leucocytozoon;* hence a good name for the syphilitic parasite would be *Leucocytozoon syphilidis.*

The suggestion that the *Spirochæta pallida* was only the male phase of a protozoon, and was not the sole cause of syphilis, met with the very greatest opposition; and in the present year (1916), with one or two exceptions,[1] [2] [3] the opposition is still as great, although not one of the observers who oppose it has tried to repeat any of the work. I cannot deal with all the theoretical arguments which have been brought forward against my discovery of the *Leucocytozoon syphilidis,* as it is not in the scope of my lectures, and they have been fully dealt with in my book, "The Biology and Treatment of Venereal Diseases."[4]

Suffice it to say here, that I have proved these contra-arguments to be false, by a study of the chemistry of the protozoon, a piece of work in which I was ably assisted by R. L. Mackenzie Wallis.[5]

As this chemical study opened new paths of research to me, some of which seemed to lead towards the explanation of the *rationale* of the Wassermann reaction, I will here indicate what they are.

I found that the phases of the *Leucocytozoon syphilidis* consisted mainly of lipoid-globulin molecules, which proved themselves to be more resistant to reagents than the lipoid-globulin molecules of the host's cells—viz., the protoplasm of the plasma cells and nucleoli. Further, that their reducing action was also greater, especially in the case of the spirochætal phase, owing to its containing in its molecule a fatty acid not completely saturated.

As a result, it was a simple matter to devise several differentiating methods of staining the organism.

Glossary.

TROPHOZOITE means a protozoal body parasitic on, and therefore nutured by, one of the cells of the host.

MEROZOITE means division of a protozoal body.

GAMETOCYTE means a cell which gives rise to a reproductive cell.

BLEPHAROPLASTS are the same as the nucleoli of nuclei.

GAMETE means the adult sexual cell.

ZYGOTE means the product of fusion between two sexual bodies.

SPOROBLAST means a body that is going to give rise to spores.

SPOROZOITE means an animal spore, in contradistinction to a vegetable spore.

REFERENCES.

[1] Peyri Rocamora (1913), "Revista de Med. Cir. y Especialidades," vii, 1.
[2] Peyri Rocamora (1913), "Treballs de la Sociétat de Biologia," i, 89.
[3] Klausner (1914), "Archiv f. Derm. u. Syph.," cxix, 214.
[4] McDonagh (1915), "The Biology and Treatment of Venereal Diseases." Harrison and Sons, London.
[5] McDonagh and Mackenzie Wallis (1914), "Biochemical Journal," vii, 517.

WORKS CONSULTED.

Ray Lankester (1903), "A Treatise on Zoology" (Part 1). A. & C. Black, London.
Schaudinn (1911), "Arbeiten Fritz Schaudinns." L. Voss, Leipzig.
Kolle u. v. Wassermann (1913), "Handbuch der pathogenen Mikroorganismen," Zweite Aufl., vii. G. Fischer, Jena.

CHAPTER III.

DYEING AND STAINING.

FOR many years controversy has been keen between advocates of physical and of chemical theories. As Bayliss[1] says: "It may be taken as established that a physical theory, based only on coefficients of partition, due to greater solubility of dyes in the tissues than in the staining solution, is inadequate."

Although facts may be brought forward in favour of the chemical theory, owing to the light which our knowledge of colloids has thrown upon the subject, the same facts can easily be explained on the physical theory. No one knows where chemistry ends and physics begins, and *vice versâ*, since in the colloidal state they are interdependent. To describe dyeing and staining as being physico-chemical phenomena would be more correct.

Almost all the members of the group of dyes are colloidal in nature, that is to say, they do not readily diffuse through animal membranes. Like other colloids, they have high molecular weights, and they are generally amorphous. In "solution" these dyes form typical colloidal suspensions, the size of the particles varying in different cases, consequently some stains appear quite homogeneous, whilst others show the well marked characters of suspensions. Further, many dyes are eliminated by filtration, and they may be separated by this means, for example, methylene blue occurs in the urine after subcutaneous injection.

Owing to their colloidal nature, the small particles of a dye in suspension become electrically charged, and, for convenience, dyes may be divided into negatively and positively charged colloids. The negatively charged colloidal particles of a dye will therefore show electrical migration to the anode, whilst positively charged colloids will travel towards the kathode. It follows from this also, that on mixing a positive dye with a negative dye, the charges will be neutralised, and the resulting colloidal mixture will then exist in an uncharged condition, *e.g.*, the eosin-methylene blue mixture, the former being negatively charged, whilst the latter is positively charged. It must be remembered that, with one or two exceptions, all dyes are neutral salts. The distinction between acid and basic dyes is that the so-called basic dyes are salts of an organic coloured base with an inorganic acid; the acid dyes, on the other hand, are salts of a coloured organic acid with an inorganic base.

The most notable feature common to all dyes is that they exhibit the phenomenon of adsorption (Bayliss[9]). By adsorption is meant a " combination " between two substances which is not strictly in the nature of a chemical union, that is to say, in which there is no direct proportionality between the concentration of the solution and the amount adsorbed. There is some kind of physico-chemical affinity between the bodies adsorbed and those which take them up, but this affinity is more of a mechanical than of a true chemical nature. To take an example, if a series of solutions of Congo red be taken, of different concentrations, and the same amount of filter paper be placed in each, a part of the dye is taken up, but in relatively larger proportions in the more dilute solutions of the dye.

There are, however, instances where the combination of the dye with the material acted upon seems to be of the nature of a true chemical combination, but these are rather exceptional cases, *e.g.*, the staining of nuclei with rongalit white. Even in this case it would probably be more correct to describe the staining as a physico-chemical action. Rongalit white is the leuco-base of methylene blue, produced by reduction, but, when it comes in contact with an oxidase system, such as is met with in nuclei, the active oxygen reconverts the colourless into a coloured base, in which condition the dye is adsorbed.

The presence of electrolytes is a most important factor in the process of preparing histological specimens. As regards their adsorptive capacities, the dyes, as a whole, are very sensitive to electrolytes, and the effect appears to be proportional to their colloidal nature. Electro-negative dyes, like Congo red, are increased in adsorptive power by the addition of kations, such as lithium, potassium, sodium, ammonium, magnesium, and calcium ions. On the other hand, anions such as hydroxyl, acetate, chloride, oxalate, sulphate, and phosphate ions depress adsorption. With electro-positive dyes, such as toluidine blue, the reverse is the case, namely, anions increase and kations depress the adsorptive capacity. The effect of electrolytes, however, is much more marked in the case of kations than in that of anions. The salts of those metals which form positively charged colloidal hydroxides—for example, iron—have a very powerful effect on the adsorptive capacity of electro-negative dyes. In every case, the ion promoting adsorption of the dye is carried down with it.

If attention be now paid to histological preparations, it is possible, in the light of these observations, to interpret the changes induced in the act of staining. In the living cell, the substance to be stained has a negative charge, for the reaction of the tissues always tends towards the alkaline side of neutrality. It is known that protein solutions, in an alkaline medium, are always negatively charged; it follows, therefore, that living cells will take up basic dyes, and that electrolytes are not essential to the process. When the cells die, the electrolytes attached to the protein constituents of the cell are split off, with the result that the cells now readily take up acid dyes. Hæmoglobin is one of the few electro-positive colloids, hence the reason why red blood corpuscles stain so readily with eosin and acid fuchsin. Further, the fixation of a dye is facilitated by heat, and this fact has been utilised in Altmann's[2] method of acid-fuchsin staining. Mayr[3] has also shown that the affinity of the Nissl bodies of nerve cells for basic dyes is destroyed by previous treatment with neutral salts. This observation further emphasizes the importance of electrolytes in the process of staining. Another reason why basic stains act better, is because, protein being negatively charged, when it becomes stained it electrolytically dissociates the stain, and, owing to its charge, it adsorbs the positive ion, which in basic dyes is the coloured one.

Further light is thrown upon this subject if the action of dyes on proteins *in vivo* be considered.

The dyes which give the best results for *in vivo* work are aqueous solutions of borax-methylene blue, polychrome methylene blue, brilliant crystal blue, Nile-blue sulphate, and alcoholic solutions of toluidine blue, thionine, and azure II. The disadvantage of the alcoholic solutions is that they must be well diluted before use, as they crystallise so readily; this is especially the case with thionine. Azure II stains deeply, but, unfortunately, it has no metachromatic properties.

The metachromatic properties of borax-methylene blue are dependent upon the basic sodium biborate. The latter acts upon methylene blue to produce both methylene violet, which, as a basic dye, shows affinity for acid substances, and methylene red, which is an acid dye, and so shows affinity for basic substances.

Borax-methylene blue, when freshly prepared, has practically no metachromatic action, but, the longer the stain is kept, the more this property increases. Finally the methylene red becomes the stronger dye, and stains the cells just as the methylene violet does. Borax-methylene blue appears to be at its best when it has been kept for a year or two. As the methylene red scarcely comes into play in the fresh solutions, no harm is done by adding 0·1 grm. eosin to 100 c.c. borax-methylene blue, for a true chemical compound results. The eosin picks out the granules in the polymorphonuclear leucocytes, and it brilliantly stains the eosinophile granules, but does not affect any one of the stages of the syphilitic organism.

From what has been stated, it will be easily seen that a description of the syphilitic organism *in vivo*, from its reactions, will entirely depend upon the characters of the borax-methylene blue which is used. Now, as impregnated female cells do not stain with eosin, or with the methylene red of freshly prepared borax-methylene blue, but only with the methylene red of borax-methylene blue which has been kept for some time, the increase in the basicity resulting from impregnation cannot be very great. It is far more probable that what change there is in reaction is in the opposite direction, and that the reason for staining with methylene red, a fact which I have frequently observed, is due to an increase in the reducing action, as will be shown later. The *Spirochæta pallida* and the female cell after impregnation are rich in hydroxyl-groups, which have a strong reducing action, therefore these phases stain with methylene red and not with methylene violet.

The sporozoites may remain for some time unstained, or they may immediately stain a dense violet. The intracellular phases stain late, and the early ones show an affinity for the methylene violet moiety, whilst the late ones, viz., the coils, take up the methylene red. The females, before fertilisation, remain unstained, except their chromatic network and blepharoplasts, which immediately stain with methylene violet. The *Spirochæta pallida* stains pink, and when it has impregnated a female cell and when the whole cell has come to a sudden standstill, a pink diffuse stain comes over the cell like a mantle. The sporozoites, while in the spore cysts, show a greater affinity for methylene red than for methylene violet, and some spore cysts are seen which stain distinctly metachromatically.

In staining *in vivo* with a negatively charged colloid, it follows that basic dyes will react best, and this has been found to be the case. The reason why neutral red, neutral violet, Bismarck brown, auramine, diazine green, malachite green, tropæolin 00, and Congo red do not give good results, is simply due to the fact that they are negatively charged dyes, and therefore they cannot stain cells, which contain colloids in " solution " with a negative charge, and which exist in a medium on the alkaline side of neutrality. It follows that good staining can be obtained only by using dyes with a positive charge, hence the reason why borax-methylene blue and polychrome methylene blue serve so admirably, for it is only under such conditions that adsorption can come into play.

The varied affinity shown by the different phases of the *Leucocytozoon syphilidis*, on the one hand for methylene violet, and on the other hand for methylene red, is due to the prevalence of a substance which has strong reducing properties (lecithin-globulin), and not so much to a change in the reaction.

I will now mention some important points brought out by staining fixed specimens with pyronin and methyl green. Both these dyes are basic, therefore positively charged colloids and influenced by anions. As is well known, methyl green shows a considerable affinity for chromatin.

Both Pappenheim[4] and Martin Heidenhain[5] explained the specific action of methyl green for chromatin, as being due to the breaking down of the weak basic salt by the strong nucleic acid radicle. The nucleus, however, did not stain with pyronin, which they regarded as being a more strongly basic salt. As a matter of fact, since methyl green is a tri-amino stain, it is by far a stronger base than the di-amino stain pyronin, the basicity of which is also diminished by its extra oxygen atom. Furthermore, acetic acid increases methyl green staining, and if acids combine with the free amino-groups of the salt, acetic acid would have done this before the nucleic acid could do so. Therefore, this explanation of its action, which had held sway for some years, cannot be the correct one.

A stain which had been largely used by Unna,[6] [7] namely, rongalit white, was found to resemble methyl green in many respects, and was only known to stain the oxygen positions of the tissues.

Rongalit white is the leuco-base of methylene blue, and it is prepared with sodium sulphite and formalin. It is, therefore, a colourless and basic mixture, and the methylene blue is only brought out as a dye in the presence of oxygen. As rongalit white stains the nuclear part of the cell, Unna concluded that methyl green also picked out the oxygen foci of the tissue, and that it was, therefore, a reduction-sensitive stain.

By a series of experiments, Unna showed that methyl green was far more sensitive to reducing substances than methylene blue, and that malachite green came in between, but that such reducing agents as grape sugar and hydroxylamine were without effect, *i.e.*, they did not decolourise methyl green. Another difference between methyl green and methylene blue and malachite green was that the leuco-bases of the last two could be reconverted into their coloured bases by the addition of hydrogen peroxide, but this could not be done in the case of methyl green.

Until Unna enunciated his theory of staining by oxidation and reduction, we were under the impression that staining depended upon reaction; in other words, that acid substances stained with basic dyes, and were therefore termed basophilic; and that basic substances stained with acid dyes, and were therefore termed acidophilic. No doubt, in part this conception is correct, but there is also no doubt that it was carried very much too far. After all, fixed protoplasm is an amphoteric substance, *i.e.*, it can act as a base or as an acid; for instance, the protoplasm of plasma cells stains well with acid fuchsin, which is an acid dye, or with pyronin, which is a basic dye. Although it stains with both, it stains better with the latter than with the former; therefore, under ordinary circumstances, it may be stated that protoplasm, using the word in a very general sense, prefers to act as an acid.

From my description of the *Leucocytozoon syphilidis*, and from the coloured plates which illustrate my book,[8] it will be seen that, by using Pappenheim's stain (a mixture of pyronin and methyl green), the syphilitic bodies stain with pyronin; but that they differ from all other cells, in that the nucleus also apparently stains with pyronin, and with a much deeper red than the rest of the cell. Working on the reaction hypothesis, or on the electrolytic theory, one must assume, then, that the protoplasm, and especially that covering the nucleus of the syphilitic organism, is strongly basophilic, and is negatively charged. It has, however, already been shown to be partly acidophilic, from the *in vivo* examinations, when it was pointed out that certain phases showed an affinity for methylene red. We have, then, a paradox, and a solution to the problem can be be found only if we adopt Unna's theory of oxidation and reduction.

Methylene violet, like methyl green, is a reduction-sensitive dye, although not to the same degree. The reason why certain phases stain with methylene red is not because the protoplasm is acidophilic, but because it has reducing properties; and, as methyl green is far more sensitive than methylene violet, every phase stains with pyronin, while only in those in which the reducing action is greatest is the affinity of methylene violet for nucleic acid overcome. The result of this is, that the reducing substance stains with methylene red. This reducing substance does not stain very readily with all acid dyes in fixed specimens, should a basic dye be present as well; because, if pyronin is supplanted by acid fuchsin, most of the nuclei of the syphilitic organisms stain with methyl green. Therefore, this characteristic pyroninophile substance of the syphilitic organisms is a strong reducing agent, is basophilic, and so is negatively charged, according to the electrolytic theory; but the action of its electric charge is overshadowed by its reducing action. Therefore, we have another extremely important factor coming into play in the act of staining.

If we now look closer into the subject of oxidation and reduction, we shall see not only why pyronin does not stain nuclei, and why methyl green does not stain the protoplasm of the plasma cells, but it will become clear that the same principle which underlies dyeing and staining underlies the immunity reactions and chemotherapy. Methyl green stains the nuclei, owing to the presence of active oxygen.

The substance which activates the oxygen is called a peroxidase, which also exists in nuclei. A peroxidase is a ferment which acts on a peroxide to produce active oxygen. The chain of events is called the oxidase system.

Peroxides are characterised by the presence of two atoms of oxygen, directly united together; they form part of the lipoid-globulin molecules, and their power of oxidation is small.

Peroxidases are enzymes, and are peculiar in that they are the active forms of metals such as iron, manganese, copper, etc., which exist in the form of hydroxides. These metals are attached to the lipoid-protein molecules as ions. The presence of a peroxidase in the lipoid-protein molecules, enables those molecules to stain with methyl green; in other words, lipoid-protein molecules containing peroxidases will adsorb methyl green, but not pyronin, owing to the presence of active oxygen, which converts pyronin into the colourless carbinol base. If the active oxygen be destroyed, as may be done by treating the sections with acids (not acetic acid), nuclei will stain with pyronin perfectly well, but not with methyl green.

The reason why methyl green will not, in the presence of pyronin, stain the syphilitic parasites, the protoplasm of plasma cells, and nucleoli, is because the lipoid-protein molecules in the bodies just mentioned contain a reducase system which masks the oxidase system.

An oxidase system consists of a peroxidase + peroxide = active oxygen, or oxidation. A reducase system consists of a reducase (Schardinger's enzymes, perhydridase, di-sulphide-protein) + perhydride oxygen = active hydrogen, or reduction. Active hydrogen reduces methyl green, and forms a leuco- or colourless base, hence it does not stain.

Pyronin, therefore, is adsorbed, provided a perhydridase instead of a peroxidase acts as the catalyst.

Staining, then, so far as methyl green and pyronin are concerned, is dependent upon the active hydrogen reducing the former to its leuco-base, and upon the active oxygen oxidising the latter to its carbinol or colourless base.

Attention should be now drawn to mordanting. Mordants are colloidgenic substances; that is to say, by various changes they give rise to colloids. Mordants react with stains by adsorption, and the adsorption is, as is the case in general, dependent upon the electric charge of the colloids adsorbed. A mordant is a substance which prepares the way for the receptivity and fixation of the dye in question.

One of the best examples is aluminium mordanting. The process begins with aluminium acetate; this salt is hydrolysed in solution into the colloid aluminium hydroxide and acetic acid. The aluminium hydroxide, having a positive charge, is adsorbed by cotton, for instance, which in contact with water has a negative charge. Such a process increases the action of acid dyes, and also fixes them in the cotton, in virtue of the tri-valent, positively charged aluminium ion, which becomes deposited upon the cotton.

Aluminium hydroxide, in the presence of protein, acts as a peroxidase, and therefore increases the amount of active oxygen. Therefore, mordanting sections which contain protein with aluminium enhances methyl green staining, and diminishes that of pyronin. From what has just been stated, and reverting back for a moment to the staining properties of methyl green and pyronin, a paradox might seemingly be said to appear. Mordanting with aluminium hydroxide increases the receptivity of negatively charged articles for acid dyes, but it also along with its analogue in the body, namely, ferric hydroxide, increases the receptivity of nuclei for methyl green, in spite of the fact that aluminium hydroxide, ferric hydroxide and methyl green are all positively charged. The explanation is, that ferric hydroxide and aluminium hydroxide, acting as they do in the presence of protein as peroxidases, form active oxygen, and that it is the active oxygen which increases the receptivity of nuclei for methyl green. Ferric hydroxide and aluminium hydroxide, acting as mordants, where no protein is present, increase the receptivity of negatively charged substances for acid dyes, because they do not act as peroxidases, and because the tri-valent positive metallic ions are stronger than the mono-valent hydroxyl (OH') ions. So far as pyronin is concerned, this difficulty does not at first sight arise, since the di-sulphides are acidic, and therefore have a negative charge, while pyronin is basic, and has a positive charge. A paradox only arises when it is noted that the result of the reducase action is active hydrogen. Active hydrogen is a kation, and has a positive charge. Therefore, the relationship which exists between active oxygen and methyl green does not exist between active hydrogen and pyronin. Methyl green will be adsorbed where active oxygen is present, because of the negative charge that active oxygen possesses, and because methyl green is a positively charged dye. Pyronin, being also a positively charged dye, will only be adsorbed by a group possessing the opposite charge, since in staining fixed tissue electrolytes are all important. As in the oxidase-reducase system the only groups not heretofore mentioned which possess a negative charge are the hydroxyl groups (OH′), it is reasonable to assume that pyronin becomes adsorbed by these groups. This view is further strengthened by the fact that peroxide hydrogen (H_2O_2 or $OH+OH$) is, perhaps, the most important substance formed by the reducase system. Iodine, used as a mordant, increases the receptivity of cells for pyronin, owing to the peroxide it forms with hydrogen and hydroxyl ions.

Alkalis, and therefore ferric hydroxide, or, rather, the active oxygen that results from its action on a peroxide, convert pyronin into its colourless carbinol base, hence lipoid-proteins, in which the oxidase system is more to the fore than the reducase system (and this is the case with nuclei), stain with methyl green, and cannot stain with a dye which is oxidation-sensitive. In the same way lipoid-protein molecules, in which the reducase system is more to the fore, stain readily with pyronin, and cannot stain with a dye which is reduction-sensitive, since such a dye is converted into its leuco-base by the active hydrogen.

Dyes which are neither oxidation- nor reduction-sensitive stain both the nuclei and the protoplasm of plasma cells, though, speaking generally, nuclei will not stain with acid dyes, while the protoplasm will do so. The reason why nuclei will not stain with acid dyes is doubtless due to the nucleic acid radicle. Therefore, although reaction does play a part in staining, oxidation and reduction are very important factors, which have not been considered fully before. They are also phenomena which bring staining into line with immunity and chemotherapy.

REFERENCES.

[1] Bayliss (1915), "Principles of General Physiology." Longmans, Green & Co., London.

[2] Altmann (1894), "Die Elementarorganismen," 2te Aufl. Veit, Leipzig.

[3] Mayr (1906), "Beitr. chem. physiol. Pathol. (Hofmeister)," vii, 548.

[4] Pappenheim (1901), "Virchow's Archiv," clxvi, 424.

[5] Heidenhain (1888), "Zeitschr. f. wiss. Mikr.," v, 519.

[6] Unna (1913), "Biochemie der Haut." G. Fischer, Jena.

[7] Unna u. Golodetz (1912), "Die Bedeutung des Sauerstoffs in der Färberei." L. Voss, Leipzig.

[8] McDonagh (1915), "The Biology and Treatment of Venereal Diseases." Harrison & Sons, London.

[9] Bayliss (1906), "Biochem. Journ.," i, 175.

CHAPTER IV.

A SKETCH OF THE MAIN THEORIES OF IMMUNITY UP TO THE DISCOVERY OF THE WASSERMANN REACTION, WITH SPECIAL REFERENCE TO THE NATURE OF COMPLEMENT.

IMMUNITY is another word for resistance, and resistance depends upon bactericidal or protective substances, which circulate in the serum. The first to give a name to these substances was Buchner,[1 2] who called them alexines (ἀλέξειν, to ward off). The next step towards the elucidation of these protective substances was the discovery of phagocytosis by Metchnikoff,[3 4] and very soon afterwards several observers showed that leucocytes formed alexines.

At about this time, it was noted that a relationship existed between certain bactericidal and hæmolytic substances. This opened the way for vast investigation, since hæmolytic experiments were easy to carry out, and their results could be easily read.

No very satisfactory explanation as to the nature of these bactericidal and hæmolytic substances was forthcoming, until Ehrlich suggested a theory, which later became known as the "side-chain" theory. Ehrlich[5 6] supposed that when a toxine or a red blood corpuscle found their way into the body, they combined, by means of their chemical affinity, respectively with the cells which the host manufactured to combat them. It is probable that the earlier workers on this subject explained the action of alexines to themselves, as did Ehrlich afterwards, but they did not possess Ehrlich's genius for coining words to express their meaning. It must be remembered that Ehrlich never had any proof that the union between a toxine and a bactericidal substance was due to a chemical affinity, which he later called a "receptor." It is this very suggestion of chemical affinity without proof, which has for so long prevented a true understanding of the phenomena of resistance.

Further investigations soon showed that alexines consisted of two component parts—one thermostabile, the specific immune body which Bordet[7] [8] called *substance sensibilisatrice*, the other thermolabile. The name which the *substance sensibilisatrice* ultimately received, and from Ehrlich, was the immune body or antibody. Ehrlich went further, and divided the antibody again into two parts, which he called haptophore groups—the one cytophile, which fixed itself on to the cell (red blood corpuscle, bacterium, etc.), and the other which fixes itself on to the thermolabile component. The thermolabile substance was called by Ehrlich complement; therefore the other haptophore group received the name of complementophile. As the antibody thus consisted of two parts, it was called amboceptor.

It was obvious that a union, or better adsorption, took place between the cytophile part of the amboceptor and the foreign body, but no one had any proof that adsorption occurred between the other two groups.

It was known from the first that the antibody was specific, but the camps were divided as to whether complement were specific or not.

The cellular origin of the alexines now requires a little further explanation. The first observers to prove that bactericidal substances did emanate from leucocytes were Hankin and Kanthack,[9] and they showed that they came from the granules of the polymorphonuclear leucocytes. Hence these granules received the name of alexocytes.

From this time onwards, most pathologists believed that the alexines of the blood were similar to those obtained from the leucocytes, but even here there were some dissentients, chief among them being Schattenfroh,[10] whose writings should be consulted by those interested in the subject.

It must be remembered that in making extracts from leucocytes there is a great danger of extracting substances other than those required, and substances which not only do not normally exist in the serum, but which have an inhibitory effect on those substances whose homologues do circulate in the serum. It is a well-known fact that extracts of nuclei have an inhibitory effect upon the action of the protein particles in the serum.

Investigations along other lines proved that phagocytosis was dependent upon substances circulating in the serum.

Metchnikoff was fully alive to these so-called "soluble stimuli," and was of the opinion that their function was to stimulate the leucocytes towards phagocytosis. On the other hand Denys,[11] together with Leclef, Havet,[12] etc., were of the opinion that the active substances in the immune serum influenced the bacteria and not the leucocytes, and that they were absorbed by the former. This work appears to have remained in oblivion, till Wright,[13] [14] in 1903, showed that in the serum there was a labile body, which altered the bacteria in such a way that they were more easily to be ingested by the leucocytes. The labile body he called "opsonin."

A year later, Neufeld and Rimpau[15] [16] showed that certain virulent bacteria could be ingested by leucocytes, only if they had been prepared by a specific thermostabile amboceptor, which they called "tropin".

The problems of immunity became still further complicated by the discovery of agglutinins and præcipitins.

Many observers were under the impression that alexines, opsonins, bacteriotropins, amboceptors, agglutinins, præcipitins, etc., were different substances. Because the action of each is different from that of the others it does not follow that they are all separate substances, they may be only exhibitions of different physico-chemical phenomena possessed by one substance, and this, in my opinion, they are. Moreover, the study of each has usually been made outside the body, and in no one instance are the conditions at all analogous to those of the action of the resisting substance *in vivo.* It will be as well to consider, for the present, that the above words only describe different phenomena which the resisting substance is capable of exhibiting *in vitro.* Discussion was rife for some time, whether tropins, or opsonins, etc., existed naturally in the serum, or whether they were first formed, only after an infection had become implanted. As I shall attempt to show later, a general resisting substance is in constant circulation in the serum, and is ready to combat any infection on its entry into the system.

Two more points still awaited elucidation. One was, as to the nature of the resisting substance, and the other was as to its action.

It was soon shown that the resisting substance was a protein, that it was a globulin, and that, whatever action it had, the action was dependent upon the presence of salts—an important observation, as will be seen later.

Buchner always thought that the action of the resisting substance was of the nature of a ferment, a view which met with much opposition, as the action did not quite fall into line with those of the ferments best known at that time, such as lipases, proteases, etc.

One of the most important observations made was one by Kiss,[17] who noticed that, in hæmolytic experiments, hæmolysis depended not upon the absolute quantity, but upon the concentration of the complement used. This was highly suggestive, then, that if the action of the resisting substance was of a zymotic or catalytic nature, the enzyme or catalyst was the complement portion of the amboceptor.

The general impression was that complement was an enzyme, until v. Liebermann[18] suggested that hæmolysis was due to soaps, which the serum contained. Noguchi[19] held somewhat the same view, as he had found that protein-free solutions of various soaps had a bactericidal action. Landsteiner and Ehrlich[20] found that ethereal and alcoholic extracts of animal organs had a bactericidal action, and as a result of their experiments they stated that complement contained lipoid-like substances, and that, in the process of bacteriolysis, the fatty substances in the bacteria were attacked.

It was then thought that the ferment was a lipolytic one, but, as Griniew[21] had already shown, the lipolytic ferment occurring in the serum was not destroyed at a temperature lower than 65° C., while complement was well known to be destroyed at 57° C.

The next view held was that the action of the resisting substance was a purely physical one, in other words that it was a surface tension reaction.[22]

Those who require in more detail this, the most complicated chapter in the history of medical science, should consult Kolle and v. Wassermann's[23] handbook of pathogenic micro-organisms.

Before closing the chapter, I will go more fully into the work done in connection with complement, since a discussion as to its nature will be more fully dealt with later, as it forms one of the chief points I am anxious to bring out in my lectures. The nature of the resisting substance, and its mode of action, the following pages will also fully disclose.

. The first to throw any real light upon the being of complement was Ferratà,[24] who succeeded by dialysis in dividing complement into two parts. Sachs and Altmann[25] succeeding in separating these two parts, by first acidifying with weak hydrochloric acid, and then neutralising the same. Liefmann and Cohn[26] gained the same object by precipitation with carbon di-oxide. These two parts received the names of middle piece and end piece, and were so christened by Brand.[27] Brand found that the middle piece was attached to the globulin fraction of the serum, and that it was the middle piece which was fixed by the amboceptor and the red blood corpuscles in the hæmolytic experiments. The end piece remained free until the middle piece was firmly fixed, and not till then did it become bound itself.

Michaelis and Skwirsky[28] found that red blood corpuscles which had absorbed amboceptor, and had been treated with mono- and di-sodium phosphate instead of with complement, only fixed the middle piece of complement when it was added, leaving the end piece free to be collected if required. Liefmann showed that, in the Wassermann reaction, it was only the middle piece that was fixed, and that the end piece remained free in the fluid medium. Tamie Amako[29] agreed with Liefmann in so far as specific complement-fixation tests were concerned, but was of the opinion that, in the non-specific tests, not only the middle piece, but the end piece as well, was fixed. This resulted in Tamie Amako regarding the Wassermann reaction as a specific complement-fixation test.

Discussion then arose as to the nature of these two pieces, and the first light to be thrown on them was that by Sachs and Bolkowska,[30] who pointed out that both pieces were thermolabile. Then Sachs and Teruuchi[31] showed that, if guinea-pig's serum was inactivated by distilled water, further addition of sodium chloride failed to restore its action. Adding to such an inactivated serum some end piece, often had the effect of restoring the complement action, adding some middle piece, seldom did, while adding both, always did. Liefmann and Cohn, as a result of several experiments, found that the quantity of middle piece fixed was infinitesimal, an observation which led them to suggest that complement was a ferment. Fränkel,[32] using carbon di-oxide to separate the middle and end pieces of complement, found that the end piece, or, as he called it, the albumin portion, increased the action of both split and intact complement. The middle piece, or, as the same observer called it, the globulin portion, inhibited the action of both split and intact complement.

From the work of the observers mentioned, and others who have confirmed them, it may be noted that the action of complement is that of a ferment, and that complement can be divided into two parts, both of which, when added, will always restore its action.

REFERENCES.

[1] Buchner (1889), "Centralbl. f. Bakter.," v, 817 ; *ibid.*, vi, 1.

[2] Buchner (1891), "Arch. f. Hyg.," x, 727.

[3] Metchnikoff (1901), "L'immunité dans les maladies infectieuses." Masson, Paris.

[4] Metchnikoff (1913), "Handbuch des pathogenen Mikroorganismen," 2te Aufl., ii, 655. Fischer, Jena.

[5] Ehrlich (1898), "Deutsch. med. Woch.," xxiv, 597.

[6] Ehrlich (1904), "Gesammelte Schriften." Hirschwald, Berlin.

[7] Bordet (1894), "Ann. de la soc. de sc. méd. et nat. de Bruxelles," iv, 455.

[8] Bordet (1895), "Ann. de l'inst. Pasteur," ix, 462.

[9] Hankin u. Kanthack (1892), "Centralbl. f. Bakt.," xii, 777 and 809.

[10] Schattenfroh (1897), "Arch. f. Hyg.," xxxi, 1 ; *ibid.* (1899), xxxv, 135.

[11] Denys et Leclef (1895), "La Cellule," xi, 175.

[12] Denys et Havet (1894), "La Cellule," x, 7.

[13] Wright (1903), "Brit. Med. Journ.," i, 1069.

[14] Wright (1909), "Studies in Immunisation." Constable, London.

[15] Neufeld u. Rimpau (1903), "Deutsch. med. Woch.," xxx, 1458.

[16] Neufeld (1913), "Handbuch der pathogenen Mikroorganismen," 2te Aufl., ii, 401. Fischer, Jena.

[17] Kiss (1909), "Zeitschr. f. Immunitätsforschung," iii, 558 ; *ibid.* (1910), iv, 703.

[18] v. Liebermann (1907), "Arch. f. Hyg.," lxii, 277, 290, 297, 299, 306, 315, 322, 328.

[19] Noguchi (1907), "Bioch. Zeitschr.," vi, 172.

[20] Landsteiner u. Ehrlich (1907), "Centr. f. Bakt.," xlv (1te Ab., Orig.), 247.

[21] Griniew (1909), "Charkow. med. Journ.," vii, 201.

[22] Traube (1908), "Bioch. Zeitschr.," x, 380.

[23] Kolle u. v. Wassermann (1911–13), "Handbuch der pathogenen Mikroorganismen," 2te Aufl. G. Fischer, Jena.

[24] Ferrata (1907), "Berl. klin. Woch.," xliv, 366.

[25] Sachs u. Altmann (1908), "Berl. klin. Woch.," xlv, 522.

[26] Liefmann u. Cohn (1910), "Zeitschr. f. Immunitätsforschung," vii, 669 ; *ibid.*, viii, 58.

[27] Brand (1907), "Berl. klin. Woch.," xliv, 1075.

[28] Michaelis u. Skwirsky (1910), "Zeitschr. f. Immunitätsforschung," iv, 357, 629.

[29] Tamie Amako (1911), "Zeitschr. f. Immunitätsforschung," viii, 168.

[30] Sachs u. Bolkowska (1910), "Zeitschr. f. Immunitätsforschung," vii, 784.

[31] Teruuchi (1907), "Berl. klin. Woch.," xliv, 467, 520, 602.

[32] Fränkel (1911), "Zeitschr. f. Immunitätsforschung," viii, 781.

CHAPTER V.

THE WASSERMANN REACTION.

(The Non-Specific Complement-Fixation Test).

My initiation into the Wassermann reaction took place early in 1908. In 1912,[1] as the result of some work I had done, dealing with the significance of the reaction and the way in which it was influenced by treatment, I was struck by the paradoxical results which were so often obtained after patients had been well treated. After a long course of salvarsan, irrespective of whether mercury was being prescribed or not, if the blood was tested monthly, one month it would be positive, the next negative, and so on, but often quite irregularly. Since, moreover, the strongest reaction was often to be obtained in cases in which there were no spirochætæ, and where there was every evidence that the patient no longer suffered from syphilis, I saw that the reaction could not be interpreted as it had been, and set to work to unravel its *modus operandi.*

As the result of my investigations,[2] I found that the Wassermann reaction was a physical reaction depending upon certain physical properties which syphilitic sera naturally possessed, but which ordinary sera could easily be made to possess; that it was not in any way a specific reaction; and that a positive reaction did not necessarily signify that the patient had active syphilis, or that he required further treatment. The points which the investigation brought out must be dealt with now, as it is upon them that my future work upon chemotherapy is based. As there are four factors concerned in the Wassermann reaction, viz., (1) antigen, (2) complement, (3) antibody, (4) hæmolytic system, I will deal with each separately, as that will render more lucid the points I want to bring out.

Antigen.—This is a short name for the tissue extract used in place of the bacterial emulsion. It really means "that which gives rise to the formation of anti-substance."

The antigen must contain nitrogen, but not necessarily in the form of an amino-acid, as a trace of formalin increases the antigenic properties, and diminishes the amino-content by about half.

The reaction may be represented as follows:

$$H.COH + R.NH_2 = R.N.CH_2 + H_2O.$$

Adding formalin increases the size of the colloidal particles, and adding cholesterol to the antigen increases its adsorptive capacity, hence the syphilitic antigen depends for its action upon (1) nitrogen, (2) the physical state of its colloidal particles.

Antibody.—As it is not a true antibody with which we are dealing, it is better to call the substance in the serum which "combines" with the complement and antigen the reacting substance, or "reagin" for short.

The reagin proved to be a lipoid-globulin.

The reaction was found to depend not only upon how many uncombined amino- and carboxyl-groups there were attached to the molecules, but also upon whether the lipoid-globulin substance was itself richer in amino- or carboxyl-groups. The richer the molecules were in carboxyl-groups, the greater was their size—exactly what was noticed in the case of the antigen.

Temperature had an effect in altering the size of the reagin molecule, so did the time during which it was left between withdrawing the blood from the patient and testing it. Reagin was also susceptible to changes of atmospheric pressure, and was largely influenced by the addition of such substances as barium sulphate, kaolin, colloidal silicic acid, and ferric hydroxide.

Any factor which increased the size of the reagin molecules increased the positivity of the reaction, and, as temperature, time, etc., acted differently on various sera, it is quite easy to see how a non-syphilitic serum can give a positive reaction, and that a positive serum need not necessarily mean that the patient has active syphilis.

If sera were frozen quickly and for a very short time, it did not alter the Wassermann reaction, but if they were frozen for longer, and more than once, the reaction was always markedly positive. If a serum was frozen several times, the freezing point tended to become lower, and the Wassermann reaction more positive.

The factor upon which the freezing point of a serum is dependent is the concentration of the free salts, and the lower the freezing point becomes, the greater the concentration of the free salts.

If the free salts are increased as the result of freezing, it shows that they were in the first instance attached to the lipoïd-protein molecules. If they are freed from the lipoid-protein molecules, it shows that the lipoid-protein molecules have increased in size or become partially precipitated. It is evident now that we are dealing with a colloidal reaction, and that the reaction is dependent upon the size of the colloidal particles.

Shaking sera with the reagents mentioned above, threw considerable light upon the nature of the reaction.

Barium sulphate increased reagin action; kaolin had a similar action, but to a much less degree, while colloidal silicic acid and ferric hydroxide had no action.

Barium sulphate is non-colloidal; kaolin ($H_2O,Al_2O_3,2SiO_2$) is only partially colloidal. Colloidal silicic acid is $Si(OH)_4$, and is strongly colloidal, and so is ferric hydroxide, which is $Fe(OH)_3$.

Therefore the degree of activity varies inversely as the colloidal nature of the body added. Barium sulphate and kaolin act as slight protein-precipitants, a fact which can be proved by measuring the surface tension with a stalagmometer.

Although the colloidal bodies form adsorption compounds with the protein molecules, and no doubt increase the size of the colloidal protein particles, the particles are not precipitated, but still remain in suspension. It follows, therefore, that the reagin is more active as its particles change their colloidal state. As this cannot possibly be the case with a syphilitic serum *in vivo*, it shows at once that the Wassermann reaction cannot in any way give an indication as to what is happening in the body.

The only other point that I need mention about the reagin here, is that that which circulates in the blood comes from the lymphocytes, and that in the cerebro-spinal fluid emanates mainly from the cells of the choroid plexuses, and that, in both cases, it is a lipoid-globulin complex.

Complement.—My investigations led me to believe that what was called complement was part and parcel of the antibody, that it was a ferment or catalyst, and that it was probably linked on to the lipoid-globulin molecules by means of the ions.

Further investigations proved not only that complement was part of the oxidase system only as I had previously thought, but that it was a part of the reducase system as well, or, in other words, that it represented the balance between the hydroxyl and hydrogen ion concentration of both the internal (oxidase-reducase system) and the external phases.

The Wassermann reaction then is an adsorption reaction, and the so-called complement is the ferment or catalyst which stimulates, accelerates, and renders possible certain changes which result from the adsorption between the reagin and the antigen particles.

Hæmolytic System.—Blood corpuscles become hæmolysed in the presence of complement and a specific antibody. The red blood corpuscles of a sheep are used, and the antibody or, as it is sometimes called, "amboceptor," is the inactivated serum of a rabbit which has been immunised against the red corpuscles in question.

If the serum is used active and fresh, there is no need to add complement.

For the blood corpuscles to be hæmolysed, it is necessary that the serum should come from an immunised animal, therefore the reaction is specific. Upon what specificity depends will be discussed later, but what I am anxious to point out here is, that a specific immune serum is not necessary for the production of hæmolysis, since I have shown that its place can be taken by colloidal aluminium hydroxide. A non-specific hæmolytic system such as this, is, to my mind, analogous to the reagin system in the first part of the Wassermann reaction.

The other points which my work on the *rationale* of the Wassermann reaction brought out, can be more or less tabulated, and the significance attaching to each dealt with in its proper place.

The lipoid-protein colloidal particles are larger in syphilitic than in normal sera, and their adsorptive capacity is greater. I have already pointed out that the phenomenon of adsorption depends partly upon the size of the colloidal particles which become adsorbed ; therefore, as the last statement is dependent upon the first, it is only necessary to give the proof that the lipoid-protein colloidal particles are larger in syphilis.

The total nitrogen value is greater in syphilitic than in normal sera, the ultra-microscopic particles are larger, the ions are more firmly attached to the lipoid-globulin molecules, there are more salts in the ionised condition, and there are more adsorbed amino-groups.

The colloidal particles are larger in the late cases of syphilis than in the early ones, and their adsorptive capacity is likewise more marked. The increased size is due to an increase of the carboxyl-groups.

Therefore, the Wassermann reaction is more marked in late than in early syphilis; note that it is most marked in the stage during which there is the fewest number of organisms.

The more fatty the lipoid-globulin molecules become, the more unsatisfied groups (carboxyl-groups) they are likely to have attached to them. As, under certain circumstances, an unsatisfied carboxyl-group may prevent powerful reagin molecules from giving a positive reaction, owing to the influence it exerts on surface tension, it often happens that a serum from a late case of syphilis gives a negative reaction. This is also probably the reason why an extract of *Spirochætæ pallidæ* does not form an effective antigen. The lipoid-globulin or reagin molecules come from the lymphocytes, and it appears that their production, if continued over a certain time, goes on in spite of the fact that the original cause thereof has long since vanished.

As the lipoid-globulin molecules are responsible for the

Wassermann reaction, it can be clearly seen that a positive reaction does not necessarily mean that the patient has active syphilis.

If the production be over-stimulated, the cells undergo a metamorphosis, their nucleoli behave parasitically, and malignant disease is the result. I must refer those who are interested in the ætiology of malignant disease to Part II of my book, "The Biology and Treatment of Venereal Diseases."[2]

The mere fact that an antigen is not absolutely necessary, at once rules out the Wassermann reaction as being a specific reaction, but from this it does not follow that the reagin molecules, when in the body, have no specific action. Indeed, *in corpore,* the reagin must have a specific action, and upon what this action depends, a study of the Abderhalden reaction will throw some light. The serum of a pregnant woman will break down placental extract — according to Abderhalden[3] —owing to the presence of a specific proteolytic ferment in the serum. It will necessarily follow that the placental extract, for the sera of pregnant women, will also be specific. From this it follows that specificity can lie in the peptones, polypeptides, amino-acids and amines. There is a limit to the number of these substances, but there is no limit to specificity. There is also no limit to the variation of the physical configurations of the molecules of the above substances, although there is a limit to their chemical molecular configurations. Therefore there would appear to be some relationship between specificity and the physical configuration of the foundation molecule, upon which are built up the other atoms which go to constitute the lipoid-protein molecules.

Hence a chemico-physical homology exists between the molecules of the serum and those of the placental extract.

For the serum of a pregnant woman to break down placental extract, it is necessary that the serum should be fresh, or, in other words, should contain complement.

Interpreted, this means that a serum cannot break down its specific antigen readily, unless its molecules are ionised, and unless they exist in a medium in which at the start its normal hydrogen ion concentration is maintained.

I have already shown that complement represents the normal hydrogen ion concentration of the internal (lipoid-globulin particles) and external phases, therefore it is the lipoid-globulin molecules in the sera of pregnant women which break down the placental extract. Hence an analogy exists between these molecules and the reagin molecules in syphilitic sera.

The action of reagin is one of adsorption. Therefore, on the same principle, there is no reason why adsorption should not take place between the lipoid-globulin particles of the sera of pregnant women and those of the placental extract.

Reagin, *in vitro,* adsorbs the antigen, and in the presence of complement precipitation occurs, with the destruction of the complement. That which corresponds to reagin in Abderhalden's test, viz., the serum of a pregnant woman, will only adsorb an antigen (placental extract) which contains amino-groups, since a variation in the physical configuration of molecules can only affect those of proteins, or those upon which proteins are built up.

Adsorption of homologous molecules results in precipitation, and further dialysis results in hydrolysis. If complement be dialysed, its action is destroyed; and it is so, too, with reagin, owing to the fact that the lipoid-globulin molecules split up. In Abderhalden's test, my view is that adsorption between homologous molecules takes place, and this results in their precipitation and subsequent hydrolysis owing to dialysation, which allows sufficient amino-acid to get through the fish bladder to give a positive ninhydrin reaction.

At first sight, it looks as if Abderhalden's test is specific, but not infrequently a syphilitic serum from a non-pregnant woman will, with placental extract, give a positive ninhydrin reaction.

The fact that a syphilitic serum will give a positive ninhydrin reaction with placental extract, to my mind, throws a great deal of light upon both the Abderhalden and Wassermann reactions. There is a close similarity between the sera of pregnant women and syphilitic women, because women seem to be very little affected by—and some even to be immune from—syphilitic symptoms. Unfortunately, syphilitic sera will give a positive ninhydrin reaction with an extract of almost any organ.

Therefore, it looks very much as if neither of these two reactions is specific, but that they are merely group reactions, which depend upon certain physical conditions of the lipoid-protein molecules, and do not simulate the processes which take place in the body.

That hæmoglobin becomes free when complement and a specific amboceptor are present, is no proof that the red blood corpuscles have been broken down by proteolytic ferments; it is only a proof that the permeability of the colloidal membrane has been altered, and a change of the surface tension brought about. Syphilitic sera without any extract at all, provided complement be present, will sometimes give a positive ninhydrin reaction, which can be explained in this way: complement may be fixed by reagin in the Wassermann reaction, without there being any antigen present. This is due to the fact that when complement is added, not only complement, but complement plus lipoid-globulin molecules are added, and these lipoid-globulin molecules become adsorbed, acting as they do as an antigen, and, as a body in which the balance between the hydroxyl and hydrogen ion concentration is maintained, consequently precipitation occurs, with the destruction of the complement. Precipitated complement is not necessarily destroyed. A ratio exists between the degree of precipitation and the loss of action, since, if the precipitation be only slight, the complement can be collected, and its action proved by placing it in a hæmolytic system.

Should, on the other hand, the complement be precipitated in a dialysing apparatus, hydrolysis of the precipitating substance (reagin) takes place, and the complement is completely destroyed with the result that an excess of free amino-groups is formed, and they dialyse through the fish bladder, and give a positive ninhydrin reaction. Therefore, in the Wassermann reaction we are dealing with a pure precipitation, and in Abderhalden's test with precipitation and hydrolysis.

The proofs for the statements just made are to be found in the following experiments:—

Colloidal solutions have a surface tension which alters, if the colloidal particles are precipitated. If normal sera are mixed with antigen and complement, and then tested with the stalagmometer, there is no alteration in the surface tension. If syphilitic sera are treated in the same way, there is a marked alteration of surface tension, and this proves that when reagin, antigen, and complement are mixed, a precipitation occurs, or, in other words, the colloidal state which previously existed becomes changed.

From the above it would appear that there is some relationship between surface tension and the Wassermann reaction; that such is really the case, is proved by the fact that the surface tension of sera is altered by the addition of barium sulphate, *kieselguhr*, and formalin, all of which increase the anti-complementary action of sera. The addition of colloidal silicic acid or ferric hydroxide to sera does not change the surface tension, and does not cause sera to give a positive Wassermann reaction.

Therefore, there is strong evidence in favour of the precipitation theory for the Wassermann reaction. The proof that Abderhalden's test is primarily a precipitation, and finally an hydrolysis, is shown by the fact that, if fresh syphilitic sera are dialysed, the reagin is partly destroyed, and the complement is completely destroyed.

I need only say further, that the Wassermann reaction is a purely physical reaction, that it is not specific, and that it depends upon the increased size and adsorptive capacity of the lipoid-globulin molecules in syphilitic sera. Finally, there is no evidence whatever that the breaking down of an organ extract, as witnessed in the Abderhalden test, is due to a proteolytic ferment.

Before stating how this knowledge influenced me in deciphering the *rationale* of chemotherapy as applied to syphilis, it will be as well to probe a little deeper into the problems of immunity.

REFERENCES.

[1] McDonagh (1912), "Brit. Med. Journ.," i, 1287.

[2] McDonagh (1915), "The Biology and Treatment of Venereal Diseases," (Chapter X). Harrison and Sons, London.

[3] Abderhalden (1914), "Abwehrfermente," 4te Auflage. J. Springer, Berlin.

CHAPTER VI.

IMMUNITY AND COLLOIDS.

The Size of the Particle and the Action of Amino-groups.

The two important facts to bear in mind are, that the substances concerned in immunity, such as antigens, antibodies, etc., are colloids, and that they are proteins, or lipoid-protein complexes. As the immune bodies are colloidal, it will follow that they will have an electric charge, and since they are proteins, the electric charge will be amphoteric.

The great distinction between a concentrated crystalloid solution and a colloid "solution" is the difference in the surface tension in the two cases. In the former the surface tension is greater, while in the latter it is smaller, than that of water. Where the surface tension is smaller, as it is in colloidal "solutions," the electric charge has a great influence on the behaviour of the particles suspended. Any deprivation of the electric charge of the particles suspended results in the particles increasing in size, becoming agglutinated and precipitated.

Another important fact to remember is, that antigens, such as hæmotoxines, bacteriotoxines, etc., have the same characters as the substances which resist them (antibodies), in fact, they have the same stereo-chemical molecular configuration, and it is this homology which accounts for the specificity.

Now, specificity, contrary to general belief, is practically never absolute.

For instance, the Wasserman reaction may be positive in all protozoal diseases; further, salvarsan produces changes in the plasma cells, as I have described in syphilis, in other protozoal diseases, and sometimes in cases of tuberculosis, although to a much less degree. Several observers have noted that salvarsan may be beneficial in tubercular affections. An emulsion of the spores from a culture of *Sporotrichum beurmanni* can be agglutinated, not only by the serum from a case of sporotrichosis, but also by the serum from other diseases caused by fungi, for instance, actinomycosis.

At first sight, Abderhalden's test for pregnancy appears to be specific, but syphilitic sera will give positive ninhydrin reactions with placental extract.

It is interesting to note in this connection, clinically, that not only is the course of syphilis considerably modified in a woman who is pregnant, but women, in general, at any rate up to the time of the menopause, are not so severely affected by syphilis as men are.

The most rational explanation of these phenomena, to my mind, will be found in likening the arrangement of the amino-groups in the lipoid-globulin molecules to the bricks in houses.

The houses, at first sight, are always the same, but the arrangement of the bricks with which they are built varies, making each house look slightly different, when thoroughly examined. Specificity is caused by the variation in the arrangement of the bricks, which are the amino-groups in the lipoid-globulin molecules. The range of specificity is unlimited, therefore the variation in the bricks is not so much the alteration of one brick as a change in the combination of several.

This being the case, it is possible for different infections to produce partly similar combinations of the groups, which go to make up the lipoid-globulin molecules. The combination may be the same up to a point, and then the last few bricks may make all the difference.

From this it may be inferred that some of the groups which go to make up the syphilitic lipoid-globulin molecules are also the same as some of those which go to make up the lipoid-globulin molecules of the sera of women. In other words, there is a common combination of the groups somewhere, in the molecules which constitute the lipoid-globulin particles of syphilitic sera on the one hand, and the sera of women up to the menopause on the other hand, and the similarity of combination becomes the closer in women who are pregnant.

The type of colloid to which the immune bodies or proteins belong, is the type usually known as the hydrophile, lyophile, or emulsion colloid, a characteristic feature of which is the capacity for forming adsorption compounds. The resisting substance, or antibody, is the lipoid-protein of the serum, which exists in the form of an emulsion, and therefore consists of particles. These lipoid-protein particles will adsorb certain inorganic colloidal substances, such as ferric hydroxide, aluminium hydroxide, silicic acid, kaolin, etc., the adsorption being dependent upon an electro-chemical affinity between the bodies adsorbed.

At first sight, this would rather point to the fact that, when an antibody adsorbed its antigen, the adsorption would be entirely dependent upon the electro-chemical affinity existing between the two bodies, as this is the only thing common to both an antigen and to an inorganic colloid, such as aluminium hydroxide, etc. It must be remembered that an electro-chemical affinity is an affinity between oppositely charged particles. Adsorption between antigen and antibody must depend upon more than this, since a body can only be negatively or positively charged, while the variations to be met with in antigens and antibodies are infinite.

Therefore, there must be another vital factor which regulates the adsorption of antigen and antibody, and that is to be found in the protein nature of the bodies concerned. One very great difference between the adsorption of protein and inorganic colloid, and protein and protein, is that, in the latter, the size of the particle is such an important factor. Antibody will adsorb more antigen in the warm than in the cold, because in the former condition the lipoid-protein particles are bigger than in the latter condition, and because they approach nearer to the suspensoid state, a state in which inorganic colloids exist. Apart from the importance which the size of the lipoid-globulin particles have in the Wassermann reaction, this increase in their size on warming explains why, in intrathecal injections of salvarsanised serum, serum which has been heated for an hour at 57° C. has a greater therapeutic action than an unheated serum.

Distilled water increases the size of the lipoid-globulin particles, owing to the fact that it causes partial de-ionisation thereof. It might be expected, therefore, that intravenous injections of large quantities of distilled water would have some therapeutic action in those diseases which were combatted by lipoid-globulin molecules possessing naturally a large size. Such is the case, and it explains the beneficial effect which Stephens[21] [22] has said to follow injections of distilled water in syphilis. Naturally the beneficial effect is only minimal, consequently injections of distilled water can scarcely be recommended as a routine method of treatment.

The lipoid-protein particles begin to increase in size in the serum after it has been withdrawn from the body. This is doubtless the *rationale* of the good effect produced by withdrawing serum, and then injecting it again.

Freezing serum and altering the atmospheric pressure at which it is kept, have the same effect as the heating above described.

A consideration of the size of the lipoid-protein particles has thrown much light upon immunity as a whole; for one thing, it has practically drawn a line between bacterial and protozoal diseases. The distinction is not absolute, since sera from certain cases of leprosy behave like protozoal sera.

In all protozoal diseases, the lipoid-globulin particles are increased in size; in all protozoal diseases, the blood may give a positive Wassermann reaction, and, as I have shown, the Wassermann reaction is a purely physical reaction, entirely dependent upon the size of the lipoid-protein molecules, or as they are here called the "reagin" particles.

That resistance also depends somewhat upon the size of the lipoid-protein particles, the following observation will show. The more chronic a protozoal disease becomes, and probably, for that matter, a bacterial disease as well, the greater grows the size of the lipoid-protein particles.

The increase of size in the lipoid-globulin particles in chronic or old-standing cases of syphilis, is due to the greater preponderance of the carboxyl- over the amino-groups, than in the early cases.

An increase of carboxyl-groups is responsible for the

Wassermann reaction being stronger in the late cases of syphilis, but that does not mean that, *in vivo*, such lipoid-protein molecules have a greater adsorptive capacity—indeed the contrary might quite likely be the case, since adsorption is mainly dependent upon the amino-groups and it is not certain whether the increase of the carboxyl-groups is at the expense of the amino-groups or not.

Once the protective cells (mainly lymphocytes) have become accustomed to form lipoid-protein particles containing an excess of carboxyl-groups, they will go on doing so, either so long as the infection lasts, or even after it has vanished, until such a time that the carboxyl-groups are in such an excess that the cell becomes a foreign body, and behaves like a parasite upon its host. One of the most important things that happens, when there begins to be an excess of carboxyl-groups, is that certain salts disappear and the lipoid-protein molecules become less ionised, and more approaching to a suspensoid. The increase of the carboxyl-groups, the fact that the colloidal particles become less ionised and more suspensoid in character, is probably the fundamental explanation of the origin of malignant disease. From this it is obvious that the carboxyl-groups are not the most important in the lipoid-protein molecules.

The lipoid-protein molecules in the antigen used in the Wassermann reaction can be increased in size, and the positivity of the reaction thereby increased, by the addition of formalin. Formalin changes the amino-groups into methylene imino-groups, as seen from this equation—

$$\mathrm{H.COH + R.NH_2 = R.N.CH_2 + H_2O.}$$

If formalin be added to an emulsion of gonococci to be used as an antigen in the gonococcal complement fixation test, a specific test, and therefore unlike the Wassermann reaction, its antigenic properties are in such a way altered that the test ceases to be specific.

This alone shows that the important groups in the lipoid-protein molecules are the amino-groups, and that specificity is dependent upon these groups.

If vaccines be sterilised with formalin, their therapeutic action is diminished, and bacteria which have been killed with formalin will not agglutinate so well as they would have done if formalin had not been used.

If formalin be added to salvarsanised serum which has been prepared for intrathecal administration, its therapeutic action is practically *nil*, while its toxic action is considerably enhanced.

The further importance of the amino-groups will be seen when the subject of chemotherapy is discussed.

Before proceeding to the next section, there are one or two other points relative to the size of the colloidal particles which are important to mention. If the colloidal particles are increased in size by the physical means above mentioned, it signifies that some electrical dissociation of the ions has occurred on the surface, a change which brings the particles nearer to their iso-electric point, at which point they are precipitated. Such an increase in size also signifies that the surface tension has been altered, and that the normal hydrogen ion concentration of the serum has been disturbed. Such particles will, if given a chance, tend to right themselves. In the complement-fixation test, such a chance is given, by adding fresh guinea-pig's serum (complement). If the particles are sufficiently big, or the electrical dissociation of the ions at the surface has been marked enough, the complement will be adsorbed owing to its perfect ionic condition, and such an adsorption will naturally destroy such complement. Should, on the other hand, the particles themselves be perfectly ionised, irrespective of their size, they may not adsorb complement. This is the reason why fresh sera often give feeble agglutination reactions, and why active syphilitic sera in the Wassermann reaction sometimes fail to give a positive reaction.

COMPLEMENT: OXIDATION AND REDUCTION.

However different most immunity reactions may appear to be, they are alike in one point, and that is that the serum used must be fresh. If the serum is inactivated, *i.e.*, heated to 57° C., to destroy its thermolabile constituent, it is necessary to replace it by fresh serum from another animal. This thermolabile substance is usually called "complement."

Now, complement is not only destroyed by heat; freezing serum will destroy it, so will the keeping of serum under abnormal atmospheric pressure, etc. The destruction of complement is always concomitant with an alteration in the colloidal state of the lipoid-globulin particles. Complement disappears, if the particles just referred to increase in size, or become less ionised—two changes which are interdependent, when the increase in size occurs, only after the blood has been withdrawn from the circulation.

In a few late cases of syphilis, when fresh serum is withdrawn, it is found to be complement-free; the same is the case with serum withdrawn just before death, and occasionally with serum withdrawn when the patient is deeply narcosed. Complement also vanishes during anaphylactic shock.

The lipoid-globulin particles in some late cases of syphilis are in a less ionised condition; the same happens just before death, or if the patient is under the influence of a narcotic, especially if the narcotic is chloroform. This is probably the reason why sudden death under chloroform sometimes occurs. From these few remarks alone, it will be seen that complement is intimately connected with the ions of the lipoid-protein particles.

These lipoid-protein particles are emulsoid particles, occurring in a fluid medium, which mainly consists of water.

The action of these lipoid-protein particles, when acting as a resisting substance, is one of adsorption.

The process of adsorption is dependent upon oxygen, as are most reactions in which energy is involved, therefore the colloidal particles will always be avid for oxygen, which they can get only from the fluid medium, in which they are in "solution." Therefore there will be a constant oxidation and reduction.

From the work of Bach and Chodat[2 3 4 5] it would appear that active oxygen is formed through the agency of enzymes, which they call peroxidases, on peroxides, since the peroxides, as found in the living cells, have not a sufficiently high oxidation potential of their own.

Peroxidases can easily be detected, as they give marked colour reactions with certain substances in the presence of a peroxide, such as hydrogen peroxide.

An extract from the root of the horse-radish will oxidise guaiacum, with the production of a blue colour, in the presence of hydrogen peroxide. One of the most interesting points about peroxidases is that metals in a certain form have the same action. The metals which were tried at first were iron, manganese, and copper; therefore, it would look as if peroxidases were the active forms of one of these metals.

For metals to act well as peroxidases they should be capable of existing in two states of different valency.

Artificial peroxidases can be made, as Dony-Hénault[6 7] first showed—50 c.c. of water were taken, to which was added 1 grm. manganese formate, 0·4 grm. sodium bicarbonate and 10 grm. gum arabic, the whole was then precipitated by alcohol, the precipitate redissolved in water and again precipitated by alcohol. The compound formed was manganese hydroxide with an emulsion colloid—gum arabic. From work done with the other metals above mentioned, it appeared that it was necessary for them to be in the form of hydroxides before they would act as peroxidases. Existing as hydroxides, they are colloidal, and their activity is due to their colloidal state.

If the colloidal state is of so much importance, it is clear that the activity of peroxidases must be in direct relationship to the extent of the surface, or to the size of the particles, hence the need for the presence of an emulsion colloid (gum arabic, albumin, etc.). Such stable colloids as these (gum arabic, etc.) protect suspension colloids, such as manganese hydroxide, from precipitation by electrolytes. These few remarks show how necessary it is for the resisting substance to be an emulsion colloid. They also show that the greater the size of the particles the greater will be their action. If the particles are bigger in protozoal diseases than bacterial diseases, it is obvious that protozoal infections must be more severe than bacterial infections, and that therapeutic agents, since they act in virtue of their oxidising and reducing properties, must be more efficacious in the former, but that a continuation of their administration would be expected to influence the latter infections.

Realising that the lipoid-globulin particles circulated in water (fluid part of serum), it was only natural to suppose that a continued oxidation and reduction would occur between the two. As the lipoid-globulin particles have a function to perform, it is reasonable to expect that they should have peroxidases attached to them. Serum gives all the peroxidase reactions, but not after inactivation, therefore I thought it possible that complement was part of the oxidase system, especially as the knowledge already existing suggested that complement was an enzyme and a catalyst. As a result of further research, complement was found to represent not only the oxidase system but the reducase system as well, and, in addition, the normal hydrogen ion concentration of the fluid part of the serum.

There are some other points in connection with complement which should be mentioned here. When a serum is inactivated, its surface tension is altered, and its normal hydrogen ion concentration is upset. The change in surface tension can easily be rectified by the addition of an oleate, and the balance between the hydroxyl and hydrogen ion concentrations of the serum can be restored by the addition of a weak solution of an acid. Therefore, carrying out these two points should restore the action of complement.

From experiments undertaken to judge the influence of fatty acids on the Wassermann reaction, I found that the addition of an oleate would frequently prevent a positive serum from giving a positive reaction, and I[19] stated, in my article on the *modus operandi* of the Wassermann reaction, that the trace of a free unsaturated fatty acid was probably the reason why a serum from a late case of syphilis failed to give a positive reaction. Normal sera, the surface tension of which I had altered in such a way as to make them give a positive Wassermann reaction, failed to do so when the surface tension was raised by the addition of an oleate.

A serum which has been inactivated, and thus has had its hydroxyl ion concentration increased, can be restored by the addition of very dilute solutions of an acid. Too much acid destroys it again, and then it can be restored by the addition of very dilute solutions of an alkali.

Complement is destroyed during an anaphylactic shock, which usually has the action of increasing the hydrogen ion concentration of the serum. Such an inactivated serum can be restored by the addition of very dilute solutions of an alkali.

From this, complement would appear to be not only part of the oxidase-reducase system, but to represent the catalysts hydroxyl (OH') and hydrogen ($H^{\cdot}$) as well.

Any change which upsets the normal balance between these two catalysts destroys complement. Therefore, complement represents part of the oxidase-reducase system of the internal phase (lipoid-globulin molecules) and the normal hydrogen ion concentration of the external phase (the liquid part of the serum).

One of the proofs that the theory just enunciated is correct, is that " complement " can be kept, provided high concentrations of certain salts are added to it. Adding such salts to an emulsoid in "solution," prevents the normal concentration of the hydrogen ion being altered, owing to the influence they have in stabilising the permeability of the particles in the internal phase.

The complement action of a serum has usually disappeared within 48 hours; if a 20 per cent. solution of sodium chloride is added to it when fresh, the serum will retain its complement action for 14 days or more.

The great proof, to my mind, that complement forms part of the lipoid-globulin particles, and does not only represent the normal hydrogen ion concentration of the external phase, is the following fact: If the serum of a guinea-pig be dialysed, the lipoid-globulin molecules become precipitated. If the precipitate is dissolved in saline, it has no complement action, nor has the fluid which has dialysed through; but if both are mixed the complement action is restored.

Light is now thrown on the so-called middle and end pieces of complement. The middle piece is part of the oxidase-reducase system adsorbed by the internal phase, while the end piece represents the hydrogen and hydroxyl ions of the external phase or, more correct to say, the normal hydrogen ion concentration.

If complement be divided into its two pieces, the end piece alone may act feebly as complement, while the middle piece may act in the opposite direction, but when both pieces are mixed, full complement action is again restored—a fact which clearly proves the statement I made earlier, namely, that the normal hydrogen ion concentrations of both the internal phase and the external phase are interdependent.

The part played by enzymes in reduction is probably a minor one, so far as they influence immunity reactions. If the lipoid-globulin particles get oxygen from the water in which they are suspended, there must be a formation of active hydrogen. Bach[3] describes a ferment which he calls "perhydridase," which gives rise to active hydrogen, by acting upon perhydride oxygen, as a peroxidase gives rise to active oxygen by acting upon peroxide hydrogen.

So far as reactions outside the body are concerned, for a reducing ferment, or, as it is called, a reducase, to act, there must be an aldehyde present, but such an action is certainly not represented *in corpore*. My own work inclines me to take the view that the reducase system acts as follows. The perhydridase is a di-sulphide-protein which acts upon perhydride oxygen to form active hydrogen, peroxide hydrogen, and mercaptan. In the oxidase system, the peroxidase is a metallic hydroxide which acts upon peroxide hydrogen to form active oxygen.

In the reducase system, sulphur—a non-metal—takes the place of iron—a metal. The metal becomes attached to the hydroxyl-group, and the non-metal to the hydrogen-group. There must be a balance between the action of the di-sulphide-group on the one hand and the ferric hydroxide-group on the other, and this signifies a balance between the hydrogen and hydroxyl ion concentrations of the serum. Therefore, in this seemingly complicated subject, a simple explanation is afforded.

The reason why I am here laying particular stress upon oxidases and reducases is, not only because they form the basis of chemotherapy, and play such an important *rôle* in immunity, but because investigation has shown me that the phases of the *Leucocytozoon syphilidis* are particularly rich in both these ferments, proving at once that these phases are not products of cell degeneration. The plasma cell consists of a nucleus and protoplasm. The former is the main seat for the oxidases, and the latter for the reducases.

The nucleus contains no reducases except the lipoid-globulin envelope of the nucleolus. Under the lipoid-globulin " envelope " of the nucleolus, oxidases are prominent.

The phases of the *Leucocytozoon syphilidis* resemble nucleoli, since oxidases are present in abundance, but they appear to be covered by especially powerful reducases. Whether there really is a covering or not, it is impossible to say, since the oxidases and reducases cannot be demonstrated in a parasitic cell at the same time. It is also impossible to say whether the reducases be external to the oxidases, or whether both are upon the same plane. Anyhow, there is a continual oxidation and reduction going on, thereby making the phases of the *Leucocytozoon syphilidis* almost exact counterparts to the lipoid-globulin particles of the serum, since both must obtain at least a portion of their oxygen from the water in the fluid medium in which they exist.

It is probable that, in some respects, the oxidases are more important than the reducases, since the development of the cell is absolutely dependent upon the presence of active oxygen, which is used, for the greater part, by the nuclei, hence the reason why reducases appear side by side with oxidases in the nucleolus, the most active and vital part of the cell. The reducases, in the phases of the *Leucocytozoon syphilidis*, bear the same relationship to the oxidases as they do in nucleoli, hence again positive proof that the cells I take to be parasitic are, indeed, very vital cells.

Seeing how important is active oxygen, it may be readily understood how seriously the cell would be affected by the slightest deprivation of it.

As already stated, the plasma cells and the phases of the *Leucocytozoon syphilidis* resemble the lipoid-globulin particles in the serum, with one marked difference, which is, that in the latter there is no counterpart to the nuclei in the former. What purpose, then, can the active oxygen serve? Obviously only as the exciter of the lipoid-protein particles, in other words as the force or stimulator of the action the particles have to perform. The action is one of adsorption, hence the active oxygen acts as a catalyst. It is possible to go even further than this. Complement is not absolutely necessary for adsorption to occur, since adsorption will occur between aluminium hydroxide and protein (red blood corpuscles), but there will be no hæmolysis unless complement is present, clearly showing that alteration of surface tension is the result of its action.

If something analogous happens *in vivo*, where the phenomenon of adsorption is probably less marked than it is *in vitro*, and presumably not of itself sufficient to kill the parasites, it can be seen how important the so-called complement is.

Presumably, some slight alteration on the surface (a change of permeability) of the parasites takes place *in vivo*, when they are attacked by the resisting substance (lipoid-globulin *plus* oxidase-reducase system)—in any case an alteration sufficient to kill the organisms.

Action of Electrolytes.

As is well known, the action of enzymes is largely dependent upon the presence of electrolytes. Pepsin and trypsin are inactive except in acid or alkaline solutions respectively. Amylase requires neutral salts, which have also a beneficial effect, as a rule, on enzymes generally.

Agglutination, precipitation, and complement-fixation will not occur unless electrolytes are present, hence the reason why normal saline is always used as the diluent. The clotting of blood will not take place unless calcium salts are present.

Although electrolytes play a very important part in immunity reactions (indeed, complement action is void without them), their *rôle* is in a sense subordinate, as alone they have no action, and the changes produced are more dependent upon the other groups which constitute the lipoid-globulin molecules. Michaelis and Davidsohn[10] found that the precipitation phenomena in cases of some "precipitins" and "agglutinins" were nearly independent of the hydrogen ion concentration of the solution and, therefore, of the electric charge of the particles, showing that the phenomena produced were not dependent entirely upon the electrolytes. There is a reaction which has come into prominence recently, namely, the gold sol test for the cerebro-spinal fluid, which is very largely dependent upon electrolytes. This test throws considerable light upon the subject under discussion.

Gold sol is a suspension colloid, and, if a good sol is prepared, the colour is red. When such a red gold sol is added to normal cerebro-spinal fluid in different dilutions, only little change in colour occurs, but if an excess of globulin be present, as in cases of degenerative myelitis and degenerative encephalitis, the red colour changes to purple and white.

The colour of the gold sol is dependent upon the size of the suspensoid particles.[11] In red sols they vary from 6 to 30 $\mu\mu$, in purple from 30 to 100 $\mu\mu$, and in white or colourless they are too big to remain in suspension, and therefore they become precipitated. For these changes to occur. the cerebro-spinal fluid must be absolutely fresh, showing that the precipitation of the gold sol is dependent upon the electrolytes. This shows that there are more electrolytes in the cerebro-spinal fluid, and, by analogy, in the serum of actively syphilitic patients, than is the case normally.

Moreover, these electrolytes are adsorbed by the lipoid-protein particles, as I showed in my estimation of the ratio between the free and the combined sodium chloride in syphilitic and in normal lymphatic glands. In both, there was the same amount of free sodium chloride, but in the case of syphilis there was an excess of combined sodium chloride. This again proves that the lipoid-globulin particles in syphilitic sera are larger than in normal sera.

If there is the same amount of free sodium chloride in the syphilitic and in the normal lymphatic gland, it must be the combined electrolytes that precipitate, or better, adsorb the gold sol particles in the syphilitic cerebro-spinal fluid. Although we have no actual proof at present, evidence appears to favour the view that it is the uni-valent ion chlorine (Cl') that has this action upon the gold sol.

If albumin, glycine, agar, etc., be added to the gold sol beforehand, the gold sol, or, as it is frequently called, Lange's[12] test, will not act. It was known to Faraday[13] that the precipitating action of sodium chloride on gold sols could be prevented by the addition of a trace of "jelly." An analogy is to be found to this in my experiments with aluminium hydroxide.

If glycine or gelatin is added to aluminium hydroxide, its amboceptor action in the hæmolytic test is abolished, and the toxic effect of intravenous injections is completely annulled.

It seems well nigh certain that this protection against the action of electrolytes, conferred by an emulsoid on a suspensoid, is due to the deposition of a film of the former over the surface of the latter, thus, to all intents and purposes, converting such a suspensoid into an emulsoid. Emulsoids, in contradistinction to suspensoids, are insensitive to uni-valent ions, hence suspensoids which have been converted into emulsoids are *in vitro* adsorbed, but no further change occurs, such as an alteration of surface tension, etc. Important considerations are now opened up, some of which will be more fully discussed in the chapter on chemotherapy, but there are others which must be dealt with here.

If aluminium hydroxide fails to act as an amboceptor, when converted into an emulsoid by glycine, it would look, on the face of things, that electrolytes were most important in complement-fixation.

In complement-fixation tests, all the ingredients are emulsoids to start with, therefore, why should aluminium hydroxide, the moment it is converted into an emulsoid, fail to act?

In bacterial complement-fixation tests, the emulsoids must be comparatively speaking fresh, that is to say, electrically charged, but there are even some exceptions to this. In the case of gonorrhœa, for instance, some gonococcal antigens may be kept a very long time, long after electrical dissociation has occurred, and yet they will still give positive reactions. In the Wassermann test, normal sera may easily be made to give positive reactions, by submitting them to changes which destroy their electrolytes. When gonococcal antigens have been kept a long time and yet are still active, and when normal sera are so changed as to give a positive Wassermann reaction, what has happened is that the emulsoids have been partly converted into suspensoids, in which condition they immediately become sensitive to electrolytes, and thus are adsorbed by the lipoid-globulin particles of the guinea-pig's serum (complement) and by the patient's serum in the case of gonorrhœa, and by the antigen in the case of the Wassermann reaction. The Wassermann reaction with sera, the lipoid-globulin molecules of which have been rendered partly suspensoid, the hæmolytic test with aluminium hydroxide as a suspensoid, and the gonococcal complement-fixation test when the antigen is partly a suspensoid, are analogous and behave, as they do, because suspensoids are so susceptible to electrolytes. The true Wassermann reaction is dependent upon the facts that the lipoid-globulin molecules of syphilitic sera have an increased adsorptive capacity and that the antigen is an emulso-suspensoid. The true gonococcal complement-fixation test is dependent, not so much upon the electrolytes as upon the homologous stereo-chemical molecular configuration of the amino-groups. Indeed, if the ingredients are all perfectly emulsoid, that is to say, ionised to their full extent, a negative reaction may frequently be obtained. This shows that electrolytes are necessary in immunity reactions *in vitro*, but that the changes observed are not entirely influenced by them. The aluminium hydroxide + glycine does not cause hæmolysis, because there can be no homology between the stereo-chemical molecular configuration of the amino-groups in the aluminium hydroxide + glycine and of the amino-groups in the red blood corpuscles.

The reason why a partly suspensoid gonococcal antigen will still act in a specific manner is no doubt due to the fact, that the homology between its amino-groups and those of the antibody has not been destroyed.

From this it is clear that electrolytes are not the chief factors which regulate the immunity reactions.

Rôle Played by the Carboxyl-Groups.

Having considered the *rôle* played by the electrolytes and by the amino-groups, it now becomes necessary to discuss the part taken by the fatty acid or carboxyl-groups.

The word " lipoid " has appeared very largely of late years, in all work dealing with immunity, and much to the detriment of advance. Strictly speaking there is no such thing as a " lipoid," and the properties assigned to this body, although they may be more dependent upon the carboxyl-groups, which are the principal groups of the compound, actually rest on the combination the carboxyl-groups form with the amino-groups, a combination which goes by the name of lipoid-globulin. Specificity, as we have already seen, is regulated by the amino-groups, not as such, but only in so far as they are combined with the other groups which go to make up the lipoid-globulin compound. Although the part played by the carboxyl-groups is now to be discussed, it must be understood that that part could not be played except for the way in which the groups are combined with other groups. In short, all phenomena described here are dependent upon a complex body, called lipoid-globulin, and each part of the complex is only dealt with separately, as no other way of explaining its manifold actions is possible. From Overton's[14 15 16] work, the function of " lipoid " is to regulate permeability, in other words, he maintained that no substance that was not a "lipoid" solvent, could enter a cell. Overton considered that " lipoids " formed a membrane to all cells, and his theory was partly based upon the observation that this membrane was impermeable to acid dyes, but not to basic dyes. In the first place it is only natural, if the membrane consists mainly of carboxyl-groups, which have a negative charge, that they should " combine " only with substances of an opposite charge, such as basic dyes. Therefore, from this one point alone, permeability would seem to depend more upon electrical forces than upon whether substances were " lipoid " solvents or not. So far as a cell-membrane is concerned, the idea is a good one, provided it is only looked upon as an inseparable film covering the outer surface of the protoplasm. If such a film covers cells, it will cover the lipoid-globulin particles of the serum, hence bringing once again these particles into line with cells, an analogy which allows so much light to be thrown upon them.

The most satisfactory way to look upon this so-called membrane is, I think, that of Bayliss,[1] who regards it as a local concentration of integral parts of the cell protoplasm. This explanation serves, as it shows that there are many other substances than carboxyl-groups, which regulate permeability. Overton[14 15] supported his hypothesis, that the cell-membrane was essentially of a "lipoid" nature, by a large amount of powerful evidence, which goes to show the importance of the carboxyl-groups, but we have since learnt that permeability does not depend solely upon "lipoid" solvency. It is a well known fact that saponin causes hæmolysis, and also that hæmolysis occurs in hypotonic solutions. The important point to be noted here is, that the more resistant a particular kind of corpuscle is towards saponin the more sensitive it is to a hypotonic solution, and *vice versâ*. From this it is clear that the constituent acted upon by saponin is of a different nature from that acted upon by hypotonic solutions.

Again, as pointed out by Bayliss,[1] while most "lipoid" solvents do, as a matter of fact, cause hæmolysis, the absence of an effect on the part of pure olein, while a mere trace of an oleate is sufficient, shows that the solvent action exerted on the "lipoids" of the cell membrane is not the chief factor. A surface tension and adsorption effect is rather suggested, leading to modifications in the colloidal state of the membrane.

As a result of much experimental evidence, it may be said that the membrane is neither protein nor "lipoid" alone, but merely a complex colloidal system of all the cell constituents, together with those of the outer liquid (electrolytes), which diminish the surface energy at the interspace. The "lipoid" parts of the complex play a large *rôle* in regulating permeability, and their action is merely one of surface adsorption. An important point to remember is that the membrane is not an invariable permanent structure, its permeability being easily influenced by external reagents, as in the experiments of Osterhout[17] which show that sodium salts make it permeable to the sodium ion, and that the addition of calcium re-establishes the normal state of semi-permeability. This action of calcium, which is so important, will be more fully considered in the chapter dealing with anaphylaxis (Chapter VIII).

From this it would appear that electrolytes play a great part in regulating the permeability of the membrane, and doubtless they do so.

The cell-membrane, under normal conditions, acts as a non-conductor, a fact which suggests not only that electrolytes are very important, but that the primary act in adsorption takes place in the interspace. It is well known how difficult it is to stain *in vivo*, and even when cells are in the act of dying, they only appear to stain on the very surface. In fixed specimens staining again is really only a surface condensation. Although evidence can be brought forward which suggests that the carboxyl-groups play a larger *rôle* than the amino-groups, it would appear that as to whether a substance was permeable or not depended primarily upon the electrolytes in the interspace. The importance of the electrolytes has already been shown in other ways, and doubtless they bear the same relationship to the amino-groups since they regulate specificity, as they do to the carboxyl-groups since they regulate permeability.

We know that the phenomena observed in interaction between antigen and antibody *in vitro* do not occur *in vivo*, and, from the way cells stain *in vivo*, being killed by the dye as they may be, no microscopic difference is noticeable, it is clear that the interaction between the lipoid-globulin particles of the serum and the lipoid-globulin particles of the parasite is primarily dependent upon the action of electrolytes in the interspace.

The first action of adsorption, then, must be between the electrolytes of the lipoid-globulin particles of the serum and those of the host, and for this action to be of any use there must be some destruction of the electrolytes caused, or perhaps an unequal balance produced. I cannot help thinking that the first change affects the sodium chloride, since the balance of sodium chloride is regulated by the calcium salts. Aluminium hydroxide is precipitated at once by sodium chloride *in vitro*, and probably by the same salt *in vivo*, with the result in the latter instance that the animal dies. If aluminium hydroxide is converted into an emulsion colloid, it is not precipitated by sodium chloride, because emulsion colloids are insensitive to uni-valent ions, with the result that the animal does not die. If calcium chloride is injected intramuscularly before aluminium hydroxide is injected intravenously, the animal does not die, because the excess of calcium salt increases the stability of the lipoid-globulin colloidal particles and prevents the loss of the combined sodium chloride, which would otherwise occur. The mere upset, then, of the sodium chloride balance would be sufficient to alter the permeability of the parasitic lipoid-globulin molecules. The alteration is presumably insufficient to kill the parasite, since evidence has already been produced to show the importance of the amino-groups, upon which specificity appears mainly to depend.

McClendon[18] made the interesting observation that the membrane becomes considerably more permeable to electrolytes, evidenced by the increase of electrical conductivity of a mass of eggs of the sea urchin on fertilisation. As a result of experiments on the staining properties of the *Leucocytozoon syphilidis in vivo*, I showed that the *Spirochæta pallida* stained well, and that, after impregnation, the staining properties of the female gamete increased. The *Spirochæta pallida* has in its lipoid-globulin molecular constitution a fatty acid-group which is less saturated than the fatty acid-groups of the other phases, evidenced by its increased reducing action. This unsaturated carboxyl-group comes to the fore again after impregnation, since the reducing action of the female cell is enhanced by fertilisation. The *Spirochæta pallida* and the fertilised female cell are the phases most quickly destroyed by chemotherapeutic agents. Therefore, from McClendon's observation with the above, it may be said that increased permeability means increased reducing action, and increased reducing action means more fatty acids in the unsaturated state.

Increased permeability, then, is an important condition, and upon it largely depends the destruction of the parasite. I have already stated that the lipoid-globulin particles in the late stages of syphilis are more fatty than those in the early stages, the reason being that an increase of carboxyl-groups renders the particles more permeable, with the result that specific adsorption between the protective lipoid-globulin particles and those of the parasite takes place more readily, with a quicker destruction of the parasite, and that chemotherapeutic agents are more readily adsorbed. The explanation is now forthcoming as to why the synthetic anti-syphilitic remedies act so much quicker in late than in early syphilis.

INDIVIDUAL IMMUNITY REACTIONS.

From what has been said of the complement-fixation test, it is clear that there are two kinds: (1) specific, (2) non-specific.

The specific test is the bacterial complement-fixation test, in which the antigen is an emulsion of bacteria, the active part of which are the amino-groups, which possess the same stereochemical molecular configuration as those of the antibody. The antigen is adsorbed by the antibody, at a rate which is accelerated by the catalyst complement. In the process of adsorption an alteration of the surface of the lipoid-globulin molecules occurs, their permeability is affected, and electrical dissociation of the ions occurs at the surface, which has the effect of destroying the complement; hence no catalyst exists to accelerate the action of adsorption between the amboceptor and the red blood corpuscles, with the result that there is no hæmolysis in the second half of the experiment. The specificity of the test lies in the arrangement of the amino-groups in the antigen and antibody.

The non-specific test is the Wassermann reaction, in which the catalyst, or complement, is destroyed by the electrical dissociation of the ions, which occurs on the surface of the lipoid-globulin or reagin particles, which, owing to their size and increased adsorptive capacity in all protozoal diseases, especially in syphilis, are capable of adsorbing almost any colloid, which, for convenience sake in this case, goes by the name of "antigen."

There is a marked difference between the two tests, to which I have not yet referred, and that is the not infrequent occurrence of amphoterism or self-fixation, especially in the non-specific test. Self-fixation is a term applied to the phenomenon when complement gets destroyed by the antibody without the intervention of the antigen.

The *rationale* of it is probably as follows: In inactivating the serum containing the antibody, the complement is destroyed, the size of the lipoid-globulin particles is increased, and they are rendered less ionised, and more like a suspensoid.

To take the place of the destroyed complement, the fresh serum from another animal is taken.

Complement signifies a normal hydrogen ion concentration, and therefore is only part of the lipoid-globulin particles. When the serum of a guinea-pig is added, complement + lipoid-globulin particles are added, not complement alone. Owing to the great adsorptive capacity of the reagin particles, these superfluous lipoid-globulin molecules act as an antigen.

The specific complement-fixation test is a physico-chemical reaction, while the non-specific test is solely a physical reaction.

If we now take the hæmolytic system, we shall find that the tests concerned are both specific and non-specific.

The specific test is the one in which complement is destroyed, in the same way as already described, when adsorption takes place between the specific antibody (amboceptor) and the specific antigen (red blood corpuscles). Owing to the alteration of surface tension produced, a change in the permeability of the red blood corpuscles occurs, with the result that the hæmoglobin becomes free; in other words, hæmolysis occurs.

The non-specific test is the one in which complement is destroyed when adsorption takes place between an inorganic colloid, such as silicic acid[9] and aluminium hydroxide, and the red blood corpuscles, the result being the same as in the specific test.

The former is analogous to the specific complement-fixation test, the latter to the Wassermann reaction.

In agglutination and precipitation tests, we find, again, that they can be divided into specific and non-specific reactions.

Gelatin[11] forms an excellent agglutinin for typhoid and cholera bacilli. Inorganic colloids act splendidly as hæmagglutinins, provided serum is not present. Inorganic colloids will also agglutinate bacteria.

This action of serum, in preventing agglutination, is very interesting. The inorganic colloids are suspension colloids, while proteins and other hydrophile colloids are emulsion colloids. Emulsion colloids protect suspension colloids from being precipitated by electrolytes.

In the hæmolytic system, if aluminium hydroxide has been treated beforehand with gelatin or glycine, it will fail to act as an amboceptor, exactly what happens if the hydroxide is treated with serum in the agglutination experiments.

Hence inorganic colloids act in these immunity reactions by adsorption, adsorption results in electrical dissociation of the ions, and these precipitate the bodies adsorbed.

Treating the suspension colloid beforehand with an emulsion colloid does not prevent adsorption, but it does prevent precipitation, as it has rendered the electrolytes inactive.

The Analogy of Immunity to Staining.

The point where all these immunity reactions simulate staining is in the part which quantity plays; in other words, there is no direct proportionality between the concentration of the solution and the amount adsorbed. To take an example in staining, if a series of solutions of Congo red be taken, of different concentrations, and the same amount of filter paper be placed in each, a part of the dye is taken up, but in relatively larger proportions in the more dilute solutions of the dye.

The same thing happens in immunity reactions; for instance, in increasing agglutinin concentrations the amount adsorbed absolutely increases, but relatively the quantity adsorbed decreases. We find the same in complement-fixation tests, and it is especially noticeable in the non-specific tests in which inorganic colloids are used. In the production of hæmolysis with aluminium hydroxide + serum, an excess of either the one or the other will prevent hæmolysis from occurring.

This disproportionality probably accounts for some negative reactions, when positive ones would be expected, and possibly explains why in very severe infections the reactions may be negative—*vide* negative Widal in bad cases of typhoid.

It is not uncommon to find that high concentrations of an agglutinating serum will not give such good agglutination as weaker concentrations of the same serum. The same phenomenon is to be observed in the complement-fixation and precipitin tests. The respective names given to the substance which is supposed to inhibit the action in these high concentrations are agglutinoids, complementoids, and precipitinoids. A similar phenomenon is common to all colloidal reactions.

What probably happens is, that the colloidal particles are sufficiently laden with electrolytes so that they can hold no more, since any action which robs such particles of its electrolytes gets rid of the so-called inhibitory effect.

Take, for instance, a complementoid serum. So far as the Wassermann reaction is concerned, complementoid sera are to be met with most frequently in the early part of the generalisation stage—what is to be expected if my explanation is correct.

Keeping such a serum, heating it, freezing it, altering the atmospheric pressure at which it is kept, adding barium sulphate to it, all these convert such a serum into one which gives a strong positive reaction. Now all these factors rob the colloidal particles of some of their electrolytes. This is probably the reason why, when sera are used active, that negative results are obtained, but when inactivated they give a strong positive reaction.

NATURAL AND ARTIFICIAL OR ACQUIRED IMMUNITY.

There are many examples of natural immunity. For instance, cold-blooded animals are immune to infection by organisms which produce diseases in warm-blooded animals, and *vice versâ*. In any case, bacterial infections are more common in warm-blooded than in cold-blooded animals.

Diseases of birds, such as psittacosis, etc., cannot be conveyed to mammals.

Furthermore, carnivorous animals are more or less immune to those bacteria which cause diseases in herbivorous animals or in man, who subsists on a mixed diet. Man appears to be absolutely immune to rinderpest, and all but the higher mammals to such diseases as scarlet fever and measles.

Natural immunity, such as above described, is really only an apparent immunity, and is not analogous to the immunity produced by a bygone infection.

The food on which the animal lives plays a *rôle*, also its temperature, since, if the temperature of cold-blooded animals be raised, they can develop tetanus, a disease to which they are, under ordinary circumstances, immune. There is even a difference in race resistance, a difference which, in some cases, is probably due to the food upon which the animal lives.

For example, Yorkshire pigs are more resistant than other breeds to a special form of erysipelas from which pigs suffer. The older races of cattle are more disposed to tuberculosis than the new races or species.

Again, different breeds of mice react differently to anthrax and to an infection caused by the *Micrococcus tetragenus*. So far as syphilis is concerned, it is certain that all races, irrespective of climate and food, are equally prone to be infected with it. The reason why Livingstone[23] thought that pure-born Africans were immune to syphilis was simply because the races to which he referred had never been in contact with white people, and therefore had never run the risk of catching the disease.

Before the white man settled in Uganda, syphilis was unknown there, now it is well nigh a plague amongst the natives. Monkeys can be easily infected with syphilis, but it is doubtful whether the lower mammals can. Because rabbits develop chancres and regional adenitis after subcutaneous injections of spirochætal material, and general lesions, if the spirochætal emulsion be injected intravenously, it does not follow that the rabbits have developed syphilis.

Probably, if a sufficient quantity of rinderpest infection were injected into a human being, he could be made to develop symptoms simulating the real disease.

There is no doubt that infections can be produced in man or in animals which, under prevailing conditions, have a natural immunity against such, therefore such natural immunity is probably due to certain physical conditions which naturally exist, and is not analogous to the immunity which follows an infection, an immunity probably due to the artificial production in the serum of lipoid-protein colloidal particles which possess the same stereo-chemical molecular configuration as the protein molecules of the parasites.

Broadly speaking, if an individual has had a disease, he is less likely to contract the same disease again.

The period at which the first attack is cured, that is to say whether soon or late after its onset, has an influence upon the degree of the immunity acquired.

The severity of the attack also has an influence, and so has the position in the body where the organisms are first implanted.

An acquired immunity is not absolute, since the patient can usually be infected, provided a sufficient quantity of the infective material gets into his system.

The main difference between acquired and natural immunity is that, in the former the immunity is specific, and in the latter it is not.

The points brought out from the few remarks made, show that, immunity depends upon a certain physical state of the host, upon the position where the organisms are implanted, upon the number of organisms implanted, and upon whether the molecular configuration of the lipoid-globulin particles in the serum of the host is homologous to that of the lipoid-globulin molecules of the parasite.

When a man runs the risk of becoming infected with syphilis there must always be, first of all, a struggle between the cells of that man and the spores of the leucocytozoon. If the former win, then, of course, there is no infection, and *vice versâ* if the latter do. The spores having gained a firm foothold, they will develop at their host's expense, and produce a lesion which we call a chancre. A further development of these spores will lead to a generalisation of them in the body of the host, with a result that lesions will occur all over the body. Instead of the development going further still, the tide turns in favour of the host and the number of the parasites gradually becomes lessened, with the result that the lesions disappear. The turning of the tide is due to the fact that the host's lymphocytes have been able to manufacture lipoid-globulin particles, the molecular configuration of which is homologous to that of the lipoid-globulin molecules of the parasite.

Two questions arise now: (1) What is the nature of the primary struggle? (2) What is the *modus operandi* of the turning of the tide?

From the remarks I have made already, it will be remembered that there are only two factors which come into play in the adsorption between antigen and antibody; (1) hydroxyl ion concentration (oxidase-reducase system), (2) specific amino-groups.

In the primary struggle the second factor cannot possibly play a *rôle*, since it takes some time for the amino-groups in the lipoid-globulin molecules to be arranged in a specific manner; moreover, the primary struggle is a local affair, a struggle between spore and cell, while the turning of the tide is a general affair, a struggle between the lipoid-globulin particles of the serum generally, of the plasma cells locally, and those of the parasites.

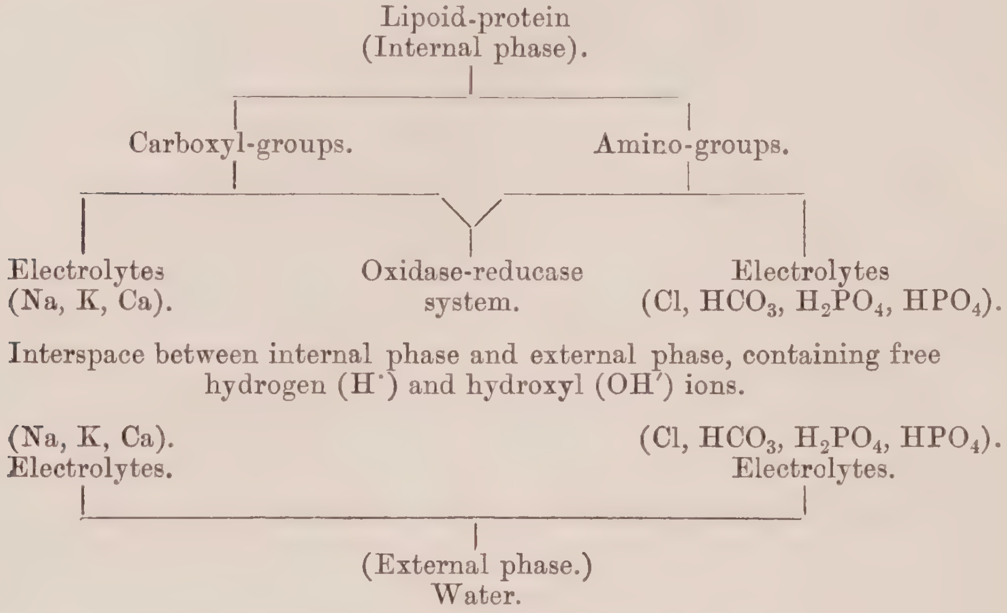
When a syphilitic spore enters the human body, to develop it must enter a connective-tissue cell or an endothelial cell. To enter a cell it must alter the surface tension of that part of the cell where it is going to enter, and this will mean a local disturbance of the normal hydrogen ion concentration. It is absolutely certain that all cells require and use oxygen, and probably certain that all cells (animal) in exchange for oxygen exhale carbon di-oxide. Should a spore be able to take some of the oxygen required by the cell it hopes to enter, the normal ratio between the oxygen intake and the carbon di-oxide excretion of that cell will cease to exist, with the result that the normal hydrogen ion concentration, or the oxidase-reducase system, will be locally deranged for the time being, with the result that the spore wins. The local surface tension of the cell will be such that the spore will be practically drawn into it. How this temporary derangement of the oxidase-reducase system can be directly prevented is not at present clear, but from analogy to experiments I have undertaken in another direction (anaphylaxis), the derangement can be prevented indirectly by increasing the stability of the permeability, which can be done by intravenous injections of calcium chloride.

The *modus operandi* of the turning of the tide is probably as follows:—

Both the lipoid-globulin particles of the syphilitic phases, and of the serum, have an oxidase-reducase system. In the case of the former, the active oxygen is used by the nucleus for propagation purposes. In the case of the latter, there being no nucleus, the active oxygen will be used for that purpose for which the particles exist, namely, for the purpose of adsorption. Since the amino-groups have the same arrangement in the two kinds of lipoid-globulin particles, the necessaries for the physical action of adsorption will be at their best. As the active oxygen in the host's lipoid-globulin molecules will only be required for this physical action, it will follow that the host's lipoid-globulin molecules have an advantage over those of the parasite, with the result that the changes which the spore in the primary struggle caused the cell it was attacking to undergo, will now be visited upon the parasites themselves. If these views are correct, we should be able to forestall the turning of the tide and increase it, by increasing the amount of active oxygen in the lipoid-globulin particles in the serum of the host. This can be done directly by (1) the administration of strongly adsorbed metallic compounds, which act as peroxidases; (2) the administration of a strongly adsorbed non-metallic compound, which acts as a di-sulphide. Here, then, we have the connecting link between immunity and chemotherapy.

RECAPITULATION.

If a rough diagram be made of the constituents of serum and each briefly discussed as it acts in overcoming the foreign body *in vivo,* and in the immunity reactions *in vitro,* the subject will, I think, be rendered clearer.


Lipoid-protein
(Internal phase).
Carboxyl-groups.
Amino-groups.
Electrolytes (Na, K, Ca).
Oxidase-reducase system.
Electrolytes (Cl, HCO_3, H_2PO_4, HPO_4).
Interspace between internal phase and external phase, containing free hydrogen ($H^{\cdot}$) and hydroxyl (OH') ions.
(Na, K, Ca). Electrolytes.
(Cl, HCO_3, H_2PO_4, HPO_4). Electrolytes.
(External phase.)
Water.

A Diagram of the Constituents of Serum.

WATER.

Water, with the salts it holds in solution, constitutes the fluid part of the serum, or as it is called the "external phase." Water itself dissociates into hydrogen and hydroxyl ions, and the constant relationship between these ions is maintained for the most part by the mono- and di-sodium phosphates and the bicarbonates. There is always a slight excess of the hydroxyl ions. The relationship between the hydrogen and the hydroxyl ions is spoken of as the normal hydrogen ion concentration.

This normal hydrogen ion concentration keeps the solid particles, *i.e.,* the lipoid-globulin molecules or, better, the internal phase, in "solution." It also forms part of the so-called complement (end-piece).

If this normal hydrogen ion concentration is upset, the internal phase attempts to restore it, which it can only do in virtue of its electrolytes. The internal phase discharges some of its electrolytes, this results in the particles increasing in size and being less in "solution." If the change is so great that the particles can no longer be kept in "solution" they are precipitated, and the point at which they are precipitated is known as the iso-electric point. So long as the particles of the internal phase remain in "solution," they behave as emulsoids, but the moment they begin to be precipitated, they behave as suspensoids. As suspensoids are very sensitive to electrolytes, once precipitation has commenced, it will continue. The conversion of emulsoids into suspensoids causes considerable alteration in the surface tension.

LIPOID-PROTEIN.

Lipoid-globulin, with its electrolytes, constitutes the internal phase. The free electrolytes, that is to the say the electrolytes which act in the interspace, are inseparable from those of the external phase. The adsorbed electrolytes are those which, so to speak, are held in reserve to become free electrolytes, should an attempt be made to render the internal phase less emulsoid. From this it will follow that, as the particles of the internal phase are bigger in some diseases than in others, for instance in protozoal diseases, the particles will have more adsorbed electrolytes, because *in vivo* the perfect emulsoid state must prevail. As the lipoid-globulin molecules are particles, they will have a surface, a surface which will vary in extent according to the size of the particle. The stability of the surface, or better to say, its permeability, is regulated for the most part by the carboxyl-groups.

The chief function of the amino-groups is adsorption, a physical phenomenon which results in the destruction of the body (parasite, etc.) adsorbed. The manner in which the amino-groups are arranged accounts for the specificity of the adsorption. Therefore, when the adsorption is specific, the phenomenon is really a chemico-physical and not a purely physical phenomenon. All functions entailing the use of energy require active oxygen. The active oxygen can be formed directly by the oxidase system, and indirectly by the reducase system, which supplies the peroxide required by the former. This oxidase-reducase system is inseparable from the electrolytes of the external phase, since it is dependent upon the hydrogen and hydroxyl ions, which prevail in the fluid part of the serum. The oxidase-reducase system forms part of the complement (middle-piece). The lipoid-protein molecules constitute the resisting substance, or the antibody, and when the parasite, that is to say the antigen, has been in the body of the host for a certain time, the stereochemical configuration of the molecules of the antibody and the antigen becomes homologous. This homology is necessary for the specific adsorption between the antibody and the antigen, as without it the total destruction of the latter is impossible.

The difference between the antibody and the antigen is, that the activated oxygen in the former is reserved for its attacking power, while in the latter it is used by the nucleus to keep division of the cell going. Therefore, taken all round, the resisting substance has the upper hand, as it must necessarily have, or else we should have ceased to be in existence. When the two bodies meet, some alteration of the concentration of the electrolytes takes place on the surface of the molecules; this results in causing an alteration of the permeability of the "membrane." The balance between the free and adsorbed electrolytes is upset, and consequently the resisting substance and the parasite perish. The parasite is killed without there being any microscopic change in its morphology. When dead, doubtless the parasite undergoes auto-oxidation.

When the antibody meets its antigen in a test tube, the same action takes place at first as occurs in the body, but owing to the supply of complement in the former being limited, the normal hydrogen ion concentration, as the result of the adsorption, soon becomes upset, and agglutination, precipitation, etc., are witnessed.

It will be better to go a little more fully into the changes which take place when an antibody meets its antigen in a test tube. First consideration will be given to the specific reactions. Adsorption occurs when an antibody meets its antigen, whether complement be present or not. If complement be not present, the change will be no more than that of agglutination. If complement be present, first agglutination, and then precipitation will occur. Without complement, practically no change in surface tension occurs, since there cannot be sufficient interchange between electrolytes to alter it. When complement is present, that is to say, a perfect emulsoid, a very great interchange between the electrolytes of the emulsoid and the partly suspensoid particles of the antibody and the antigen takes place, with the result that all the particles concerned become still more suspensoid. The more suspensoid the particles become the bigger grow the molecules, the more readily they are precipitated, and the greater the change in the surface tension that occurs.

It will follow, then, that the immunity changes witnessed *in vitro* will be agglutination, precipitation, complement-fixation (alteration of surface tension), and hydrolysis (Abderhalden's test).

This mention of hydrolysis requires a little explanation. When emulsoids are precipitated they become converted into suspensoids, but in this state they still have some electrolytes attached to them. If these electrolytes are still further removed, as they may be by dialysis, there will be a tendency for the suspensoids to behave as crystalloids. Protein which has become thus changed will be able to dialyse through a fish bladder, with the result that it can be easily detected in the fluid outside by means of ninhydrin (Abderhalden's reaction).

In non-specific reactions, adsorption will not result between antibody and antigen with or without complement, unless either the antibody or the antigen is in a sufficiently suspensoidal state, or the antibody is so big and its emulsoid characters so marked, that its adsorptive capacity is increased over and above that exhibited by ordinary emulsoids.

If either the antibody or the antigen is entirely or only in part a suspensoid, adsorption without complement will occur, but as before, for any great change in surface tension to occur, complement must be present.

The antibody is a suspensoid when the true antibody is replaced in the hæmolytic system by aluminium hydroxide or colloidal silicic acid. Without complement, the red blood corpuscles will adsorb such inorganic colloids, but no change in surface tension with resulting hæmolysis will occur, unless complement be present; because the suspensoid inorganic colloid cannot convert the emulsoid red blood corpuscles into a suspensoid state. If, even in the presence of complement, either of the inorganic colloids above mentioned is converted into an emulsoid, no adsorption with an alteration in the permeability of the red blood corpuscles will take place.

The antigen in the Wassermann reaction is an emulsion colloid, but not so perfectly emulsoid as the fresh lipoid-globulin particles in the serum, hence it will be readily adsorbed by a perfect emulsoid, provided it has sufficient surface, by an emulso-suspensoid, and by a suspensoid, with destruction of complement if it is present. Suspensoids have a bigger surface than emulsoids, any change to which the lipoid-globulin molecules of a normal serum may be subjected which makes the particles more suspensoid, such as alternate freezing and thawing, alteration of atmospheric pressure, addition of barium sulphate, etc., will result in such a serum giving a positive Wassermann reaction.

Likewise, any change in the emulsoid character of the antigen, such as treating it with formalin, etc., which produces an increase in the extent of the surface of the colloidal particles, will result in normal sera giving a positive reaction.

With what we call a normal antigen, broadly speaking, only protozoal sera give a positive Wassermann reaction, simply because of the increased adsorptive capacity of protozoal lipoid-protein molecules, or, in other words, of the greater extent of surface the particles exhibit. Any change towards the suspensoidal state of such particles, will naturally only increase the positivity of the reaction. As the lipoid-protein molecules are bigger in syphilis than in other protozoal diseases, and bigger in the late than in the early cases, it follows that the Wassermann reaction will be stronger in the former than in the latter. As the destruction of complement and the alteration of surface tension are concomitant, means which prevent the latter occurring will also prevent the former from being destroyed, but will not prevent the adsorption between the antigen and the antibody. An oleate will supply the means, and, as the carboxyl-groups are increased in the lipoid-globulin molecules in late cases of syphilis, it will be readily seen that a negative Wassermann reaction might be easily obtained with such a serum. As a lipoid-globulin particle which is fully charged will not adsorb its antigen, unless the latter is specific, the reason is forthcoming as to why syphilitic sera, when used active, will sometimes give a negative reaction, and why, even after inactivation, a serum from a case of early generalised syphilis will likewise give a negative reaction.

Sera from cases of early generalised syphilis contain lipoid-globulin particles which are very powerfully adsorbed and heavily charged, so that inactivation will rid them of only a few electrolytes, leaving sufficient to render them still so perfectly emulsoid that they are unable to adsorb a non-specific antigen.

These few remarks, I think, show how simple immunity is, and they also show that a positive Wassermann reaction neither necessarily indicates that the patient is actively syphilitic, nor that he requires treatment. In short, the Wassermann reaction is only of value when the exact colloidal state of the reagin particles is known beforehand, and such knowledge would render the reaction superfluous. The next chapter will show how that knowledge may be obtained.

REFERENCES.

[1] Bayliss (1915), "Principles of General Physiology." Longmans, Green & Co., London.

[2] Bach (1911), "Biochem. Zeitschr.," xxxi, 443.

[3] Bach (1913), Oppenheimer's "Handbuch der Biochemie," Ergänzungsband. G. Fischer, Jena.

[4] Bach u. Chodat (1903), "Chem. Ber.," xxxvi, 600.

[5] Bach u. Chodat (1904), "Chem. Ber.," xxxvi, 606.

[6] Dony-Hénault (1900), "Zeitschr. Elektrochem.," vi, 533.

[7] Dony-Hénault (1908), "Bull. Acad. Roy. Belgique," 105.

[8] Liefmann u. Cohn (1911), "Zeitschr. f. Immunitätsforschung," viii, 781.

[9] Landsteiner u. Rock (1912), "Zeitschr. f. Immunitätsforschung," xiv, 14.

[10] Michaelis u. Davidsohn (1912), "Biochem. Zeitschr.," xxxvi, 280, u. xlvii, 59.

[11] Taylor (1915), "The Chemistry of Colloids." E. Arnold, London.

[12] Lange (1912), "Zeitschr. f. Chemotherapie," i, 44.

[13] Faraday (1858), "Phil. Trans.," cxlvii, 145.

[14] Overton (1899), "Jahrbuch wiss. Bot.," xxxiv, 669.

[15] Overton (1901), "Studien über die Narkose." G. Fischer, Jena.

[16] Höber (1911), "Physikalische Chemie der Zelle u. der Gewebe." Englemann, Leipzig.

[17] Osterhout (1911, 1912), "Science," xxxiv, 187, and *ibid.*, xxxv, 112.

[18] McClendon (1910), "Amer. Journ. of Physiol.," xxvii, 240.

[19] McDonagh (1915), "Quart. Journ. of Med.," viii, 129.

[20] Michaelis u. Skwirsky (1910), "Zeitschr. f. Immunitätsforschung," iv, 357, 629.

[21] Stephens (1914), "Brit. Med. Journ.," ii, 1021.

[22] Stephens (1913), "Dublin Journ. of Medical Science," cxxxv, 15.

[23] Livingstone (1881), "Last Journeys in Central Africa." J. Murray, London.

CHAPTER VII.

A NEW AND RATIONAL TEST FOR SYPHILIS (THE EMULSOID-SUSPENSOID, OR THE E-S. TEST).

The tests to be described in this chapter are the outcome of investigations made by R. L. Mackenzie Wallis and myself, which have been published in "The Quarterly Journal of Medicine."[8]*

THE PRINCIPLES UNDERLYING THE WASSERMANN REACTION.

From a perusal of the previous chapters, it will be seen that marked differences exist between the internal phase or the lipoid-globulin molecules of syphilitic and those of normal sera, and that it is very difficult to interpret the results obtained by the Wassermann reaction correctly.

The positive Wassermann reaction given by syphilitic sera is due to the increased number and size of the lipoid-globulin molecules, their greater emulsoid character, hence their increased adsorptive capacity. The complement, which is nothing more nor less than lipoid-globulin molecules which possess a normal oxidase-reducase system, existing in a medium in which the hydrogen ion concentration's optimum is maintained, is fixed, owing to the fact that the perfect emulsoid reagin particles, after having adsorbed the imperfect emulsoid antigen particles, give up some of their ions to bring the imperfect emulsoid particles into a perfect emulsoid state. To rectify the temporary loss of ions which results from such an adsorption, the adsorbed particles rob the fully charged complement, and in so doing destroy it.

As, under normal circumstances, the syphilitic lipoid-globulin molecules are more fully ionised than the normal lipoid-globulin molecules—it is only in the early stages of syphilis that this condition exists—it will follow that such particles may be able to get the antigen particles into "solution" without calling upon the ions of the complement. It is well known that active sera obtained from cases of syphilis, in the generalisation stage, will sometimes give a negative Wassermann reaction.

---

* Publication has been delayed, and it may not appear in the journal mentioned.

This negative result, when a positive one should be obtained, is common to all immunity reactions, when fully charged lipoid-globulin molecules are employed, and it often proves a great source of trouble, when the results have to be interpreted.

In cases of late syphilis, owing to the richness of the lipoid-globulin molecules in carboxyl-groups, and to the risk of one or more of these groups being unsatisfied, a negative Wassermann reaction may be obtained, when the result should have been positive, owing to the influence such an unsatisfied group exerts upon surface tension. This is again a great source of trouble to the interpreter.

The richness of such molecules in carboxyl-groups increases their permeability, and hence their adsorptive capacity, with the result that strong positive reactions may be obtained with sera from patients who are harbouring none, or only a few organisms. Provided the lipoid-globulin molecules of normal sera are rendered sufficiently suspensoid, owing to the sensitiveness of suspensoids to uni-valent ions, they will be precipitated easily when complement is present, with the result that complement will be destroyed, and the reaction will be positive.

How is the observer to know whether his sera contain partly suspensoid particles? He cannot. Therefore he never can be sure that all his sera which give positive reactions are syphilitic sera.

As the syphilitic lipoid-globulin molecules are more numerous and more emulsoid than the normal lipoid-globulin molecules in the same bulk of serum, it struck me that it might be possible that the hydrogen ion concentration of the iso-electric point in the two cases would vary, or, if it did not, that the rate at which the iso-electric point was reached would be variable. Tests undertaken to solve this proved valueless.

Knowing that emulsoid particles contain more water than suspensoid particles, it occurred to me that if the water could be abstracted from the lipoid-globulin molecules, such molecules would become converted into suspensoids and thus become sensitive to uni-valent or multi-valent ions, which could be employed for detecting them. As a given quantity of serum contains more emulsoid particles in the case of syphilis than in a case of, at any rate, most other diseases, it was highly probable that the amount of precipitate produced by reagents would be markedly different in the two cases. Various methods were tried, with the above in view, to differentiate a normal from a syphilitic serum, with most gratifying results. I need not refer to all the methods employed, or to the steps taken which led to the best results being obtained, but will merely state that the acetic acid ammonium sulphate, acetic acid lanthanum sulphate, acetic acid thorium nitrate, and acetic acid uranium nitrate tests proved the most satisfactory.

SOME PHYSICAL AND CHEMICAL PROPERTIES OF THE SERUM PROTEINS.

The serum is generally considered to contain two proteins, namely, serum albumin and serum globulin, but it is with the latter that I am mainly concerned for the moment. It has been known for many years that, if blood serum is diluted and then slightly acidified, a portion of the protein content is precipitated; and this has been termed the serum globulin. Hammarsten[1] defined globulin as the fraction separated out by saturation with magnesium sulphate, whereas Hofmeister[2] defined globulin as the fraction separated out by half saturation with ammonium sulphate. Now it is doubtful whether the same protein is separated by these two methods. Mellanby[3] found that half saturation with ammonium sulphate removed 71 per cent. of the protein, whereas with magnesium sulphate only 47·5 per cent. was removed. Burckhardt[4] dialysed Hofmeister's protein fraction and found that a large portion of it was soluble in water. This observation led to the distinction between eu-globulin and pseudo-globulin, the latter being soluble in water, whilst the former is insoluble (Joachim and Freund[5]). These two fractions have been demonstrated in all sera investigated, also in a number of milky effusions, which are pseudo-chylous in nature (Mackenzie Wallis and Schölberg[6 7]). It has also been possible to identify these two globulins in the blood and urine in certain cases of syphilis with associated so-called "albuminuria." The solubility of the globulin in serum depends upon the concentration of the protein present, the amount of salts adsorbed, the hydrogen ion concentration, and the temperature. In addition, the amount of "lipoid" material associated with the globulin in serum produces profound alterations in its physical and chemical properties. The "lipoids" present influence the optical activity, the solubility, the electrical charge on the colloidal particles, and also the relationship of globulin to electrolytes. Under normal conditions, the proteins in the serum occur in certain definite quantities, the rate of albumin to globulin being as 1·5 : 1. This rate, however, undergoes considerable alteration in pathological sera, *e.g.*, starvation, nephritis, etc. It is not surprising, therefore, to find that, in syphilis, not only is there an increase in the amount of globulin present, but also that the lipoid-globulin complex is largely increased in this disease. The lipoid-globulin molecules are kept in "solution" by the salts present. Now, these salts confer on the serum a relative alkalinity, hence the protein particles present possess a negative charge. Such protein particles are very sensitive to the kations of any electrolyte, and they can readily be thrown out of "solution," particularly by di-basic ions. It follows, therefore, that a serum containing an excess of lecithin-globulin molecules will possess certain definite physical properties, which should render it easy of detection outside the body. These observations have guided me in searching for a test. Given an increased concentration of protein, it should be possible to so alter the salt concentration, the hydrogen ion concentration, and the temperature, as to lead to the precipitation of the whole or part of the lecithin-globulin particles, even in very small quantities of serum. For this purpose glacial acetic acid has been adopted as the reagent. The lecithin-globulin molecules in an acid solution will consist of particles possessing a positive charge, and, in consequence, the particles will be very sensitive to the anion of any electrolyte, especially the sulphate anion (SO_4''). Such a "solution" of lecithin-globulin particles in glacial acetic acid will be influenced by the addition of any electrolyte, and the hydrogen ion concentration will be reduced, in an increasing ratio, corresponding with the rising valency of the anion of the electrolyte used. The precipitation of the lecithin-globulin molecules, and also the amount of the precipitate, is sensitive to the hydrogen ion concentration around the iso-electric point, and this is brought about in the new tests by the addition of the appropriate electrolyte. The first effect of glacial acetic acid is to withdraw water from the internal phase. In this way surface tension is developed at the interfaces of the lecithin-globulin particles, which causes them to come together. The salt concentration is also considerably reduced, likewise the temperature. The addition of an electrolyte reduces the hydrogen ion concentration to the region of the iso-electric point, and the protein particles become precipitated. Knowing that definite differences exist between the lipoid-protein particles of syphilitic sera and those of normal sera, it is thus possible to distinguish between them by this means.

Technique Common to all the Tests.

Blood is taken from the vein and the serum allowed to separate off. If the serum is opaque it matters not, and a little blood mixed with it does not upset the tests. A serum will give the reaction as long as it has not undergone decomposition, consequently it may be kept several days.

When performing the tests, it is necessary to have a known negative serum, and, as well, to have a known positive serum, because the time of year and the temperature of the room make a slight difference to the readings.

For each serum four test-tubes are taken, and marked 1, 2, 3, 4. Into the first tube one drop of serum is put, into the second two drops, into the third three drops, and into the fourth four drops. The serum is not inactivated beforehand. The drop pipette should be made from narrow-bore glass tubing, so that each drop is as small as possible. As the results obtained depend upon the size of the drop used, it is, of course, imperative that the same pipette should be used for each series of tests. If the drop is the same for each series of tests, it does not matter whether in one series the drop is larger or smaller than that used in another series, provided every series contains a positive and a negative control.

The results can be read off at once, and again the next day. In the *interim* the tubes must be left at room temperature and not incubated. Incubation sends some of the precipitated protein into solution, hence it makes the variation less distinct, and the results are more difficult to read.

ACETIC ACID AMMONIUM SULPHATE TEST.

Requirements:—

1. Pipette divided into cubic centimetres.
2. Glacial acetic acid.
3. Saturated solution of ammonium sulphate.

To keep the ammonium sulphate solution saturated, it is a good plan to have the bottom of the bottle covered with crystals.

To each of the four tubes 1 c.c. of glacial acetic acid is added. The tubes become cloudy. They should then be shaken, when as a rule No. 1 tube becomes clear, No. 2 may or may not become clear, Nos. 3 and 4 remain opaque. The opacity of the tubes is always denser in syphilitic cases than in normal cases, and, in severe cases of syphilis, flocculi may form on standing, which is never the case with normal sera.

When the tubes have been well shaken a drop of the ammonium sulphate solution is added to each. The tubes are again well shaken. The density of the opacity is then noted in each tube, and the reading is taken again next day, by which time the opacity has increased.

Normal Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	0	(+)	+
Saturated solution of ammonium sulphate.	1 drop	1 drop	1 drop	1 drop
Opacity	0	0	+	+ +

Syphilitic Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	(+)	+	+ +	+ + +
Saturated solution of ammonium sulphate.	1 drop	1 drop	1 drop	1 drop
Opacity	+	+ +	+ + +	+ + + +

The glacial acetic acid robs the emulsoid particles of some of their water, and converts part of them into suspensoids, which can be detected by the naked eye, if sufficient particles are present to cause an opacity.

As suspensoids are precipitated by ammonium sulphate the degree of opacity can be enhanced.

If the opacity of the solutions is more marked in the other tubes than in the normal control tubes, it shows that the other tubes contain sera which possessed more emulsoid particles. If many sera form a series the richness of each serum in lipoid-globulin molecules can be readily gauged.

This test gives not only a picture of the state of the internal phase in a serum tested, but it serves also as a measure of the influence of treatment.

To give an instance: *Case* 1.—A patient had a primary sore and marked inguinal adenitis. There were no clinical manifestations of the generalisation stage.

The E-S. test with glacial acetic acid ammonium sulphate before and after treatment read as follows:—

Before Treatment.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	+	+ +	+ + +
Saturated solution of ammonium sulphate.	1 drop	1 drop	1 drop	1 drop
Opacity	(+)	+ (+)	+ + +	+ + + +

After Treatment.

(Four intravenous injections of galyl, and one intramuscular injection of intramine.)

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	0	(+)	+
Saturated solution of ammonium sulphate.	1 drop	1 drop	1 drop	1 drop
Opacity	0	0	+	+ (+)

Normal Serum (Control).

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	+	+	+ (+)
Saturated solution of ammonium sulphate.	1 drop	1 drop	1 drop	1 drop
Opacity	0	0	+ (+)	+ +

This result shows clearly that the amount of resisting substance in this patient's blood had been reduced to below par by treatment, and that no further treatment with the exception of mercury and iodides was indicated.

Several similar instances of a like treatment could be cited.

Every serum subjected to these new tests—and there have been 100 in all—has also had a Wassermann reaction done. As the relationship of the E-S. test with glacial acetic acid ammonium sulphate to the Wassermann reaction is the same as the E-S. tests conducted with the other acetic acid mixtures to be presently described, I will discuss it here to avoid recapitulation. Out of these 100 cases, oddly enough I had one which gave a + + + Wassermann reaction, although the patient had never had syphilis. By the E-S. tests this serum was normal.

Seventeen of the 100 cases gave a negative Wassermann reaction when the test should have been positive. By the E-S. tests all these sera were positive.

The following case will prove of interest with respect to this: *Case* 2.—A patient complained of feeling generally out of sorts for the last two or three years, and of having bouts of severe headaches. The patient had never had a chancre so far as he could remember, but on one or two occasions he had had a localised cutaneous eruption which from the description strongly suggested syphilis. On five different occasions he had a Wassermann reaction done, all with negative results, including the one I did myself. The E-S. test with glacial acetic acid ammonium sulphate was as follows :—

Patient's Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	+	+ +	+ + +
Saturated solution of ammonium sulphate.	1 drop	1 drop	1 drop	1 drop
Opacity	+	+ +	+ + +	+ + + +

Normal Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	0	+	+ (+)
Saturated solution of ammonium sulphate.	1 drop	1 drop	1 drop	1 drop
Opacity	0	(+)	+	+ +

The E-S. test being positive, I suggested a provocative injection of galyl, and the blood taken 48 hours after the injection gave a positive Wassermann reaction.

ACETIC ACID LANTHANUM SULPHATE TEST.

Requirements—

1. Pipette divided into cubic centimetres and another into tenths of a cubic centimetre.
2. Saturated solution of lanthanum sulphate in glacial acetic acid.

Lanthanum suggested itself as a good precipitant both of emulsoids and suspensoids, because it is a tri-valent metal and has a high atomic weight (139). Lanthanum sulphate is only sparingly soluble in glacial acetic acid. The E-S. test with glacial acetic acid lanthanum sulphate is carried out as follows:—

1 c.c. of glacial acetic acid is added first, the tubes well shaken, and then 0·2 c.c. of the glacial acetic acid lanthanum sulphate solution is added to each tube afterwards.

Normal Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	0	(+)	+
Solution of lanthanum sulphate in glacial acetic acid.	0·2 c.c.	0·2 c.c.	0·2 c.c.	0·2 c.c.
Opacity	flocculent precipitate	0	+	+ +

Syphilitic Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	(+)	+	+ +	+ + +
Solution of lanthanum sulphate in glacial acetic acid.	0·2 c.c.	0·2 c.c.	0·2 c.c.	0·2 c.c.
Opacity	0	+	+ + +	+ + + +

This test is very delicate and the results obtained depend entirely upon the size of the drop used. If the drop tends to be bigger than that with which the above results were obtained, an opacity may occur in the first tube of a syphilitic serum, in which case the second tube will be also opaque, and the first two tubes in the control will be clear, as seen in the following series.

Normal Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	0	(+)	+
Solution of lanthanum sulphate in glacial acetic acid.	0·2 c.c.	0·2 c.c.	0·2 c.c.	0·2 c.c.
Opacity	0	0	+ +	+ + +

Syphilitic Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	(+)	+	+ +	+ + +
Solution of lanthanum sulphate in glacial acetic acid.	0·2 c.c.	0·2 c.c.	0·2 c.c.	0·2 c.c.
Opacity	(+)	+	+ + +	+ + + +

The probable explanation of the phenomena observed is as follows. When a flocculent precipitate occurs, it shows that all the protein has been converted into suspensoid particles, in which condition they are readily precipitated by the lanthanum sulphate. When a flocculent precipitate occurs in the first tube, the second tube is clear. In the second tube sufficient of the particles have been left in the emulsoid state to neutralise the precipitating power of the lanthanum sulphate. When a flocculent precipitate occurs in the first tube containing normal serum, the corresponding tube containing syphilitic serum will be clear, and the second tube slightly opaque. There being more emulsoid particles in a syphilitic serum than in a normal serum, the first tube is clear because there are sufficient emulsoid particles left to neutralise the action of the lanthanum sulphate. The second tube is opaque because the emulsoid particles are beginning to be in excess. As the emulsoid particles are always in excess in the third and fourth tubes, whether the serum be normal or syphilitic, the two latter tubes will always be opaque, but the opacity will be more marked in those tubes which contain the greater number of emulsoid particles, *i.e.*, in those tubes containing the syphilitic serum.

ACETIC ACID THORIUM NITRATE TEST.

Requirements—Same as in the preceding test, only the lanthanum sulphate solution is replaced by a 1-per-cent. solution of thorium nitrate in glacial acetic acid.

To each tube 1 c.c. of glacial acetic acid is added, the tubes are well shaken and then 0·2 c.c. of the supernatant fluid in the thorium nitrate solution is added.

The reason why thorium was chosen was entirely due to its high atomic weight (232).

Normal Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c c.
Opacity	0	0	+	+ (+)
Solution of thorium nitrate in glacial acetic acid.	0·2 c.c.	0·2 c.c.	0·2 c.c.	0·2 c.c.
Opacity	flocculent precipitate	0	+ (+)	+ + (+)

Syphilitic Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	(+)	+	+ +	+ + +
Solution of thorium nitrate in glacial acetic acid.	0·2 c.c.	0·2 c.c.	0·2 c.c.	0·2 c.c.
Opacity	flocculent precipitate	+	+ + (+)	+ + + (+)

The results obtained with thorium are seen to be very similar to those obtained with lanthanum, but the test is not quite so delicate, the reason being that less thorium nitrate goes into solution in glacial acetic acid than lanthanum sulphate, that thorium is not a tri-valent element, and that according to the Hofmeister's series the precipitating power of the nitrate anion (NO_3) is less than that of the sulphate anion (SO_4). Thorium certainly has a higher atomic weight than lanthanum, but this point in its favour is outweighed by the greater solubility, the higher valency of the lanthanum salt, and by the fact that the precipitating power of the sulphate ion is greater than that of the nitrate ion in an acid solution.

ACETIC ACID URANIUM NITRATE TEST.

Requirements—Same as in the preceding test, but a 1-per-cent. solution of uranium nitrate in glacial acetic acid is used instead of thorium nitrate. Uranium was used because it has a high atomic weight (238) and is more soluble in glacial acetic acid than either lanthanum or thorium.

Normal Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	0	0	+	+ (+)
Solution of uranium nitrate in glacial acetic acid.	0·2 c.c.	0·2 c.c.	0·2 c.c.	0 2 c.c.
Opacity	flocculent precipitate	(+)	+ (+)	+ + (+)

Syphilitic Serum.

Tube	1.	2.	3.	4.
Serum	1 drop	2 drops	3 drops	4 drops
Glacial acetic acid	1 c.c.	1 c.c.	1 c.c.	1 c.c.
Opacity	(+)	(+)	+ +	+ + +
Solution of uranium nitrate in glacial acetic acid.	0·2 c.c.	0·2 c.c.	0 2 c.c.	0·2 c.c.
Opacity	flocculent precipitate	+	+ + (+)	+ + + (+)

The remarks made concerning the lesser precipitating power of thorium nitrate than that of lanthanum sulphate apply equally well to uranium nitrate. Uranium has a higher atomic weight than lanthanum, but it is not a tri-valent metal and the precipitating power of the nitrate anion is less than that of the sulphate anion in an acid solution.

The readings just given, obtained with lanthanum, thorium, and uranium, were made 24 hours after these salts had been added. If to such a series of tests one drop of a saturated solution of ammonium sulphate be added, most striking and immediate changes take place. In normal sera only a few crystals appear, while in syphilitic sera several crystals separate out, especially in the lanthanum and thorium tubes.

I have not ascertained the exact nature of these crystals but as to whether many form or only a few is probably due to the concentration of the glacial acetic acid, and to the number of salts set free as the result of its denaturating effect upon the lipoid-protein particles.

There is another observation which is worth recording. The uranium solution is yellowish-green, a tint which becomes discoloured in the first tube in syphilitic sera only. The

Normal Serum.

Tube	1.	2.	3.	4.
LANTHANUM.				
Saturated solution of ammonium sulphate. Readings taken directly afterwards.	1 drop	1 drop	1 drop	1 drop
Crystals	(+)	(+)	+	0
THORIUM.				
Saturated solution of ammonium sulphate. Readings taken directly afterwards.	1 drop	1 drop	1 drop	1 drop
Crystals	+ +	(+)	0	0
URANIUM.				
Saturated solution of ammonium sulphate. Readings taken directly afterwards.	1 drop	1 drop	1 drop	1 drop
Crystals	0	0	0	0

Syphilitic Serum.

Tube	1.	2.	3.	4.
LANTHANUM. Saturated solution of ammonium sulphate. Readings taken directly afterwards.	1 drop	1 drop	1 drop	1 drop
Crystals	+ + + +	+ + +	+ +	+ +
THORIUM. Saturated solution of ammonium sulphate. Readings taken directly afterwards.	1 drop	1 drop	1 drop	1 drop
Crystals	+ + + +	+ + + +	+ + +	0
URANIUM. Saturated solution of ammonium sulphate. Readings taken directly afterwards.	1 drop	1 drop	1 drop	1 drop
Crystals	0	0	0	0

thorium solution is colourless, but in the tests a greenish colour appears. Unlike the uranium, no change in colour is noticeable in the different sera.

CONCLUSIONS.

1. Syphilitic sera contain more lipoid-globulin molecules or emulsoid particles than normal sera.

2. Emulsoid particles are in "solution," therefore they cannot be seen with the naked eye. They are kept in "solution" by the ions attached to them and by the water their molecules contain.

3. Removal of the ions and the water, or denaturation, converts emulsoid particles into suspensoids, or in other words precipitates them, with the result that they now become visible to the naked eye.

4. As there are more emulsoid particles in syphilitic sera than in normal sera, it is obvious that a partial conversion of such particles into suspensoids will lead to varied changes, which can be detected by the naked eye.

5. Glacial acetic acid has the power of causing partial conversion of the lipoid-protein particles from one colloid state into another.

(Emulsoid → Suspensoid).

6. As suspensoids are more sensitive to electrolytes than emulsoids, the emulsoid particles which have been converted into suspensoids can be made still more evident to the naked eye by the further addition of various electrolytes, especially of the sulphate anion, as owing to the presence of glacial acetic acid the colloid particles are in an acid medium.

7. The reagents chosen were the sulphates of ammonium and lanthanum, and the nitrates of thorium and uranium.

8. The E-S. tests give an exact representation of the condition of the lipoid-globulin molecules or resisting substance. The tests are, therefore, not only invaluable so far as diagnosis is concerned, but also in so far as they can be used for regulating treatment.

9. The E-S. tests are rational, easy to manipulate, and the cost involved is negligible.

REFERENCES.

[1] Hammarsten (1880), "Pflüger's Arch. f. d. ges. Physiol.," xviii, 38.

[2] Hofmeister (1887), "Arch. f. exper. Path. u. Pharm.," xxiv, 247.

[3] Mellanby (1907–8), "Journ. of Physiol," xxxvi, 332.

[4] Burckhardt (1882), "Arch. f. exper. Path. u. Pharm.," xvi, 322.

[5] Joachim u. Freund (1902), "Zeitschr. f. physiol. Chem.," xxxvi, 407.

[6] Mackenzie Wallis and Schölberg (1910), "Quart. Journ. of Medicine," iii, 301.

[7] Mackenzie Wallis and Schölberg (1911), "Quart. Journ. of Medicine," iv, 153.

[8] McDonagh and Mackenzie Wallis (1916), "Quart. Journ. of Medicine," ix.

CHAPTER VIII.

ANAPHYLAXIS.

(DISTURBANCE OF THE NORMAL HYDROGEN ION CONCENTRATION.)

THE explanation of this phenomenon, so far as it is understood at present, throws considerable light upon immunity problems in general, and also explains the cause of sudden death which may occur after intravenous injections of certain substances, of which salvarsan is one.

The word *Anaphylaxie* was invented by the French physiologist Richet,[1] who was the first to study and to give a rational explanation of the phenomenon.

Richet found that, from the tentacles of the sea anemone, a toxine could be prepared which caused intense vascular congestion in the viscera of dogs, leading to death after some hours. He found also that, if the dose given at first was too small to kill the animal, a second dose of only one-twentieth the amount of the first was sufficient to produce serious symptoms, which resulted in death.

For death to take place after the second injection, it was necessary that a certain minimum number of days—eight to twelve—should elapse between the two injections. If, for instance, the second injection was given three or four days after the first, severe symptoms did not follow. Von Pirquet[2] described an analogous phenomenon, to which he gave the name of *Allergie*. Although we use the word *Anaphylaxie* in England, calling it "anaphylaxis," the phenomenon often goes by the name of "hypersensitiveness." Although several writers have gone to much trouble to differentiate between anaphylaxis and allergy, there can be little doubt but that anaphylaxis, allergy, and hypersensitiveness are three words for one phenomenon. It is to be hoped that these three words will soon become extinct, as the majority of the words which have been coined in medicine, during the last ten or fifteen years, should. As we proceed the clinical phenomena described will be seen to be due to a disturbance of the normal hydrogen ion concentration.

It was first thought that only proteins could produce anaphylaxis, but symptoms exactly analogous to those produced by even a non-toxic protein, such as egg-albumin, can be brought about by the intravenous administration of aluminium hydroxide to rabbits, and of salvarsan to man.

The one necessary characteristic of the substance which is to produce what is commonly called anaphylactic shock, is that it should be a colloid. Here, then, is the first indication of the connection between anaphylaxis and immunity—substances to produce anaphylactic shock must be in the colloidal state, and so must antigens, antibodies, etc.

A fact that has led to much confusion is that, in different animals the symptoms vary according to the particular organ which is the most affected, since the sensitiveness of the various organs differs in different animals. Fortunately, we now know that, however different the symptoms may be, the *modus operandi* of the anaphylactic shock is the same in every case.

Another point about the anaphylactic substance is, that it is specific ; here, again, the specificity is not absolute ; similar to the specificity met with in immunity reactions, it is only of an overlapping nature.

The current explanation of anaphylactic shock is that, owing to the adsorption between the anaphylactic substance and the protein in the serum, digestion occurs, as a result of the liberation of a proteolytic ferment. In carrying out my experiments on the action of metals in chemotherapy, I used aluminium hydroxide, which I found produced fatal shock in rabbits when injected intravenously, but not when injected intramuscularly. I found, further, that if a little glycine or gelatin were added to the colloidal hydroxide, it could be injected intravenously with impunity. Furthermore, if calcium chloride had been injected intramuscularly the day before, no anaphylactic shock followed the intravenous administration of the colloid.

I have pointed out already that aluminium hydroxide will take the place of amboceptor in a hæmolytic system, and that hæmolysis occurs because adsorption takes place between the colloid and the red blood corpuscles.

Hæmolysis will not take place unless complement is present, and, when hæmolysis does occur, it is due to an alteration of surface tension.

In the process of adsorption, the permeability of the red blood corpuscles becomes changed, and the hæmoglobin is set free.

Aluminium hydroxide is a suspension colloid, and only as a suspension colloid will it produce an anaphylactic shock and act as an amboceptor in a hæmolytic system. Aluminium hydroxide plus glycine, gelatin, or albumin, etc., is an emulsion colloid.

Suspension colloids are sensitive to electrolytes, while emulsion colloids are not, hence, as an emulsion colloid, aluminium hydroxide cannot be precipitated, with the result that the electrolytes of the lipoid-protein particles are not disturbed.

As a suspensoid, aluminium hydroxide is readily precipitated by even uni-valent ions, such as those of sodium chloride, hence it will be readily seen that the balance between the adsorbed and non-adsorbed electrolytes of the lipoid-globulin particles will be changed. This results in an alteration of the permeability of the particles, an upset of the balance between the hydrogen ion and hydroxyl ion concentration of the serum, with the result that the patient dies.

Calcium chloride increases the stability of the permeability of the lipoid-globulin molecules, hence, even if aluminium hydroxide be injected later as a suspensoid, electrolytic dissociation of the ions of the lipoid-globulin particles does not occur.

The toxic action of aluminium hydroxide is due, primarily, to its strong positive electric charge. The hydroxyl ions, OH', which are uni-valent, are cast off, leaving a positively charged tri-valent ion $Al^{\cdot\cdot\cdot}$, which is held in close proximity to the oppositely charged ions in the serum, thereby temporarily increasing the hydrogen ion concentration of the blood. The converse is also the case, as silicic acid is as toxic as aluminium hydroxide. In the case of silicic acid it is the negative ions which are held in close proximity to the oppositely charged ions in the serum, thereby temporarily increasing the hydroxyl ion concentration. If such is the explanation for substances of only one charge, how is the anaphylactic shock caused by proteins, which have a double charge, to be explained? So far as inorganic colloids are concerned, specificity plays no *rôle*, and, as I have shown that specificity depends upon the amino-groups, it will be seen that the anaphylaxis produced by proteins is due to the amino-groups, since, if these groups are in any way destroyed, anaphylaxis will not result.

In my work on the complement-fixation test, I showed that normal sera would give a positive Wassermann reaction, if the size of the lipoid-globulin particles were increased, and also if the size of the antigen particles were increased. Increasing the size of the particles referred to, is the same as saying that the particles have been robbed of some of their electrolytes, in other words, electrolytic dissociation of the ions has occurred.

Complement, as used, is lipoid-globulin fully charged, so that when it comes into contact with other lipoid-globulin molecules which are not fully charged, electrolytic dissociation of the ions of the former occurs, with the result that the complement action vanishes.

For protein to produce anaphylaxis, it must not be fresh, it must have been deprived of some of its electrolytes, hence when it is injected, provided its amino-groups have the same stereo-chemical molecular configuration as those in the lipoid-globulin molecules of the serum, adsorption will occur, and, owing to the protein being more or less de-ionised, its adsorption will immediately cause a disturbance of the balance of the electrolytes of the lipoid-globulin particles, a destruction of complement, and a rise either in the hydrogen ion or in the hydroxyl ion concentration of the blood, according to the charge of the prevailing ion of the antigen. As injected proteins are on the alkaline side of neutrality, the change will be a rise in the hydrogen ion concentration.

The rapidity with which the animal dies, clearly shows that anaphylactic shock is not due to an enzyme, producing protein digestion. Anaphylactic shock may result from an intravenous injection of any colloid, and, as I have already stated, it may occur after salvarsan and its substitution products. In my experience, it occurs more often in the early than in the late stages of syphilis; it occurs more frequently after the fourth and the subsequent injections than after the first three; it occurs more frequently if the interval between the injections is more than 10 days; it occurs more frequently with neo-salvarsan than with salvarsan, and especially with the English and French substitution products, and it occurs more frequently if the patient has had a meal just before an injection.

A few minutes after the injection, the patient suddenly becomes faint, he then vomits, becomes unconscious, the heart stops beating, and the patient may die in spite of all means tried to save him. On the other hand, he may become deeply cyanosed, the lips and tongue may swell up, and the patient appears to be at death's door. A true picture of asphyxia is produced, due to a tetanic contraction of the smooth muscle fibres of the small bronchi; when the tetanic contraction ceases, the patient regains consciousness. In the first case, the attack is primarily cardiac; and in the second case, primarily pulmonary. In the first case, when the patient regains consciousness he almost immediately begins to complain of excruciating pains in the legs and feet; a short time later he is perfectly normal. After some days a toxic erythema may or may not supervene. Some cases are followed by typical arsenical dermatitis.

The cardiac attack is usually accompanied by a very marked fall in the temperature, and, besides the vomiting, the patient may pass his motions and water; when the motions are passed in this way, it will be noticed that they are generally fluid, showing that an irritation of the intestines has been produced by the marked fall in blood-pressure. If the blood be examined, there is found to be a leucopenia, and the clotting time of the serum is delayed.

If death occurs during a pulmonary attack, *post mortem*, the lungs exhibit the picture of emphysema, with sometimes marked œdema and hæmorrhages, or merely only the results of venous congestion.

The *post-mortem* appearances of the organs in the cardiac form are very important, since the cardiac attack is more frequently met with than the pulmonary, and since there always appears to be such a mystery about these cases when they come before a Coroner's Court. There may be ecchymoses in the abdominal organs, the gall-bladder is often markedly extended, and there is almost invariably some degeneration of the kidneys.

The renal changes are those which lead to a wrong diagnosis, and one almost always reads in the pathologist's report that the patient was an unhealthy individual, and that if his kidneys had been normal, presumably he would not have died. Anaphylactic shock can occur in any individual, indeed the healthier the condition he is in, I won't say that the greater his chances are of being affected, but his chances are greater when compared with chronic alcoholics, whose kidneys may show some sign of disease before the injection is given. For all practical purposes, one may say that the kidneys are perfectly normal in by far the greater majority of cases, and that the degeneration noticed, *post mortem*, is entirely the result of the shock. To prove this it is only necessary to examine the kidneys of rabbits which have been poisoned with a toxic drug like neo-kharsivan.

Since the same substance on the same class of animal (man) can produce on the one hand a cardiac attack, and on the other hand a pulmonary attack, it shows fairly conclusively that the first change takes place in the serum, and then either the heart or lungs are attacked, and that the shock is not of central nervous origin, as has been commonly thought to be the case.

There are one or two other points connected with anaphylaxis which interest me intensely, as, in my opinion, they so strongly support some of the views I hold.

Anaphylactic shock is a condition which cannot be mistaken but there are symptoms which are frequently associated with what one might call chronic anaphylaxis, and they are very frequently mistaken or, better, misunderstood. Asthma may be a symptom of chronic anaphylaxis, *Urticaria pigmentosa* is probably always significant of a chronic anaphylaxis, and so are various other dermatoses. In anaphylactic asthma, in *Urticaria pigmentosa*, and in the cases of dermatitis I have in mind, there is always an increase of either eosinophile cells or mast cells. In anaphylactic shock, and in all cases of anaphylaxis, some change takes place in the complement, in anaphylactic shock complement vanishes altogether, and in chronic anaphylaxis complement is in part destroyed. Complement represents the balance between the hydroxyl and hydrogen ion concentration of the serum ; in anaphylaxis we know that this balance is upset. In chronic diseases, in aleucæmic lymphocytomata of syphilitic origin for instance, I have shown[10] that complement may fail to be demonstrated in serum freshly withdrawn, and in such cases eosinophile cells and mast cells are often markedly increased in the diseased areas. In my paper[3] on the *rôle* played by the lymphocyte, etc., I showed that, in any infection, be it acute or chronic, if the protective capacity of the host's cells were stimulated to its utmost point, then eosinophile cells and mast cells usually made their appearance. Elsewhere I have shown that eosinophile cells and mast cells are very rich in oxidases. Not only are the oxidases especially pronounced, but the reducases as well—note affinity of mast cell granules for the methylene red moiety of polychrome methylene blue. The oxidase-reducase system is that part of the solid particle which regulates the hydrogen ion concentration of that particle. The oxidase-reducase system corresponds to the phosphate and bicarbonate systems which maintain the constant hydrogen ion concentration of the fluid part, and in which the solid particles are in "solution." We know that the phosphate and bicarbonate systems are more easily upset than the oxidase-reducase system, and are at once influenced by the condition of anaphylaxis, hence in both acute anaphylaxis, during which death is threatened, and in the chronic condition, it would naturally be expected that the host would pour forth those cells or solid particles which are going to do their best to restore the hydrogen ion concentration back to its normal state. Hence the reason why eosinophile and mast cells are prominent in these conditions. If my theory be correct, any measure which will prevent the balance between the hydroxyl and hydrogen ion concentration of the solid particles in the serum from being upset, should cause a disappearance of the symptoms present. As the balance just referred to is regulated by the permeability of the solid particles, increasing the stability of the same should produce the result desired. Calcium chloride injected intravenously or intramuscularly does increase the stability of the permeability, and this drug (but not when given *per os*) does temporarily cause the symptoms to disappear. I have treated cases of chronic dermatitis, exfoliative dermatitis, *Dermatitis herpetiformis* and *Urticaria pigmentosa*, with injections of calcium chloride, and have noticed symptoms which had persisted for months, and even years, to disappear in a few days. As calcium chloride does not get rid of the cause, the improvement wrought is naturally only temporary. Further on it will be noticed, that the experiments I have undertaken with calcium chloride all go to support the interesting theory here maintained. Another set of experiments undertaken by Friedberger and Hartoch,[4] and by Friedberger and Langer,[11] still further goes to support the above. The first two observers found that anaphylactic shock could be avoided by injecting intravenously a hypertonic solution of sodium chloride, and the last two observers found that anaphylaxis could be prevented by stomachal administration of large quantities of sodium chloride.

Hypertonic saline preserves complement, or it prevents the normal hydrogen ion concentration from being altered, hence the explanation of the action of sodium chloride in anaphylaxis. Segale[12] was, I believe, the first to prove that in anaphylaxis the hydrogen ion concentration was raised, that the alkalinity of the serum was diminished, that the respiratory capacity for carbon di-oxide and oxygen was diminished, and that the formol-titration substances (amino-acids) were increased—points which all fit in with the views expressed.

A characteristic feature of all anaphylactic conditions is vaso-dilatation. The pronounced vaso-dilatation and fall of blood-pressure, made most observers consider that the start was due to a disturbance in the central nervous system; no one seems to have realised that the primary change is an alteration of the permeability of the lipoid-globulin particles in the serum. That the vaso-dilatation is of peripheral origin, is clearly shown by the fact that adrenalin, a drug which acts centrally, is not of very much use in anaphylaxis, and that chloride of barium, which acts peripherally on the smooth muscle fibres, causes an instantaneous rise in the blood-pressure (Biedl and Kraus,[13] and Meyer and Gottlieb[14]).

A few words are necessary about the so-called anti-anaphylaxis. By anti-anaphylaxis is meant a suddenly occurring insensitiveness to the re-injection of an anaphylaxis-producing antigen. Otto[15] was the first to show that guinea-pigs, which had been injected with a very large dose of horse serum, remained insensitive to a repetition of the antigen injection. Although several other workers have confirmed this observation, a satisfactory explanation of its action is nowhere to be found. My own opinion is that anaphylaxis is the phenomenon occurring *in vivo*, as we see in the complement-fixation test, and also in the agglutination test *in vitro*. In the two immunity tests just mentioned, it is a well-known fact that an excess of either antigen or antibody will prevent the test from acting. Both anaphylaxis and immunity tests *in vitro* exhibit certain phenomena due to an unequal balance of the electrolytes adsorbed to the bodies concerned. In other words, there is, in all the reactions referred to, an attempt on the part of the protective substances (antibodies) to bring the bodies injected (antigen) into the same perfect emulsoid state as that in which they themselves are. Such a change can only be produced, provided neither the perfectly emulsoid bodies nor those imperfectly emulsoid bodies are in excess. The proof of this is seen in the fact, that absolutely fresh or fully ionised antigen when injected does not produce anaphylaxis, and that aluminium hydroxide, if converted into an emulsion colloid, will not act as an amboceptor in a hæmolytic system, nor will it produce anaphylactic shock. The following retort might now very naturally be made, namely, if such an imperfect emulsion colloid, such as aluminium hydroxide-glycine, imperfect when compared with anaphylaxis-producing horse serum, fails to produce anaphylaxis, why should a more perfect emulsion colloid, such as horse serum, do so? The answer is perfectly simple, the reason being that in the one case specificity is playing no part—that is to say, there are no amino-groups possessing an homologous stereo-chemical molecular configuration to those of the protective substance—while in the second case specificity is playing a very big *rôle*, hence the degree of the adsorption is different in the two cases.

From the above it is only reasonable to expect that, if a repetition of the injection of the antigen is prescribed in the pre-anaphylaxis stage, then the symptoms usually produced by such an injection would not set in. We know very well from practical experience that such is the case.

A point which, I think, is in favour of my explanation of the phenomenon anti-anaphylaxis is the fact that, after a varying interval, if small doses of the antigen are re-injected, anaphylaxis occurs as before.

I should think there is little doubt but that eclampsia and the other so-called intoxications of pregnancy are symptoms of anaphylaxis; if so, it would be interesting to learn if calcium chloride and the various other suggestions here offered relieve the symptoms.

There are a few points in what has gone before which warrant a little further discussion. First, attention will be directed to the proteins, and then to the drugs.

Proteins are widely used in vaccines, and all of us know how readily vaccines may produce a marked local, general, and focal reaction, in short, a mild degree of anaphylaxis.

These reactions can be avoided by adding serum to the bacterial emulsions, preferably a specific antiserum,[8] this process is generally called sensitisation, and originated with Besredka.[5] [6] [7]

Proteins will not produce anaphylaxis *de novo;* the body must have prepared a resisting substance against each individual protein first, hence the reason why anaphylaxis, at first sight, always appears to be specific.

Proteins, however specific they may be, will not produce anaphylaxis when absolutely fresh, hence anaphylaxis is due to proteins which possess amino-groups with the same stereochemical molecular configuration as the proteins which they are sent to attack, and to proteins the molecules of which have suffered electrolytic dissociation of the ions attached to them. De-ionised specific amino-bodies are adsorbed by their ionised counterparts, the lipoid-protein molecules in the serum, with the result that the ions of the latter undergo electrolytic dissociation, and this upsetting of the hydrogen ion concentration of the blood gives rise to troublesome symptoms. The specificity is not absolute, since tuberculin will occasionally give a positive reaction in a late case of syphilis, the reaction being much more marked if an injection of "pallidin" is given afterwards, and *vice versâ*.

Almost any protein extract will produce a positive cuti-reaction in late syphilis, and I have been able to prepare certain lipoid-protein emulsions which give as good reactions as Noguchi's spirochætal extract.[9]

If a few injections of a syphilitic extract be given to a patient who has not had syphilis, time allowed to elapse, and then another injection be given, a positive cuti-reaction may be obtained. This, to my mind, clearly shows that a positive cuti-reaction in syphilis is no indication whatsoever that the patient still has the disease, or, if he has, that it is active.

So much for the proteins, now for the drugs.

Anaphylaxis is more likely to occur with the fourth or fifth injection of salvarsan than with the first three. Salvarsan, owing to its being adsorbed by the lipoid-protein molecules of the serum, causes an autolysis of the latter, with a result that, in time, the lipoid-protein molecules will not be so completely ionised as they were to start with—a fact which can be easily proved by comparing the free and the combined sodium chloride content of the serum at different periods.

If the injections are given quickly after one another anaphylaxis does not occur.

Salvarsan does not produce anaphylaxis in late syphilis, presumably because the lipoid-protein molecules, being in any case less ionised than they are in early syphilis, never reach that degree of instability which was possible earlier.

Furthermore, oxidation is more to the fore in early syphilis and reduction more to the fore in late syphilis, and as all metals show a great affinity for oxygen, and as the arsenic no doubt acts catalytically as a peroxidase, a far greater upset of the lipoid-globulin molecules might be expected to result from the administration of the salvarsan in early than in late syphilis.

Anaphylaxis has been much more common with the English and French substitution products for salvarsan, for the probable reason that there is a greater percentage of suspensoidal arsenic in the English and French products than in the German.

From the fact that anaphylaxis is much more common after the administration of suspension than of emulsion colloids, and that it occurs more frequently after the substitution products of salvarsan, one may, I think, safely presume that more arsenic exists uncombined with the amino-groups in the substitution products than in salvarsan and in neo-salvarsan. The way to avoid anaphylaxis following the arsenical preparations, is to give frequent injections spread over one single period, allowing as short an interval to elapse between each injection as possible, not to give an injection directly after a meal, and to allow the solution to mix with the serum before it is injected.

Now, then, comes the question as to how we can save the lives of those who develop anaphylactic shock, after intravenous injections of certain therapeutic agents.

The treatment of the case will naturally depend upon whether the patient has a pulmonary or a cardiac attack.

In a pulmonary case, atropine must be injected ; giving oxygen to inhale, or employing means usually used to produce artificial respiration, is useless. In a cardiac case, the patient should immediately have his head and thorax lowered, injections of ether, camphor, and strychnine should be given, oxygen should be inhaled and artificial respiration instituted. Heat to the heart region should be applied, and brandy should be taken by the patient if possible.

One of the most interesting things in connection with this subject is that an anaphylactic shock will not occur, if the patient is under ether anæsthesia: note that sudden death under chloroform is practically always to be avoided if ether is administered first.

In both the cardiac and the pulmonary cases, pituitary extract may be used, and injections of camphor and adrenalin made, but their utility is quite minimal.

From experiments I have done on rabbits, I am inclined to favour intravenous injections of calcium chloride, since I have succeeded in preventing anaphylactic shock in rabbits by timely intravenous injections of a 1 per cent. solution of the salt. Although I have not had occasion to try this on a human subject, yet, the above experimental work on animals leads me to think that such a manœuvre might possibly save the life of the patient.

REFERENCES.

[1] Richet (1912), "L'Anaphylaxie." F. Alcan, Paris.

[2] v. Pirquet (1910), "Allergie." Springer, Berlin.

[3] McDonagh (1914), "Brit. Journ. of Dermat.," xxvi, 283, 337.

[4] Friedberger u. Hartoch (1909), "Zeitschr. f. Immunitätsforschung," 581.

[5] Besredka (1902), "Ann. de l'instit. Past.," xvi, 918.

[6] Besredka (1902), "Compt. rend. de l'acad. des Sci.," cxxxiv, 1330.

[7] Besredka (1910), "Bull. de l'Instit. Past.," viii, 241.

[8] McDonagh (1915). "The Biology and Treatment of Venereal Diseases" (Chapter 39). Harrison & Sons, London.

[9] *Ibid.* (Chapter 12.)

[10] *Ibid.* (Chapter 10.)

[11] Friedberger u. Langer (1912), "Zeitschr. f. Immunitätsforschung," xv, 535.

[12] Segale (1912), "Pathologica," Nota ii, iii, No. 76.

[13] Biedl et Kraus (1910), "Arch. internat. de Physiol.," ix, 217.

[14] Meyer u. Gottlieb (1910), "Experim. Pharmakol."

[15] Otto (1912), "Zeitschr. f. Immunitätsforschung," xii, 41.

WORKS CONSULTED.

Dale (1912), "Journ. of Pharmacol. and Exper. Therapy," iv, 167.

Doerr (1913), "Handbuch der pathogenen Mikroorganismen," ii, 947, 2te Aufl. Fischer, Jena.

Michaelis (1910), "Handbuch der Biochemie," ii, 689. Fischer, Jena.

Seligmann (1913), *ibid.* Ergänzungsband, 248.

CHAPTER IX.

CHEMOTHERAPY.

It will be remembered in the "side-chain" theory that Ehrlich considered the union between antibody and antigen was due to chemical affinities which each of these bodies possessed for the other. This being so, Ehrlich conceived the idea that the principle could be applied to treatment, and the application later received the name of "chemotherapy." The principle was shortly this—

To find drugs, the chemical groups of which would combine with the chemical groups of the parasites to be attacked, without combining with the chemical groups of the host's cells. The chemical groups of the drugs received the name of "chemoceptors." The disease which Ehrlich hoped to combat was sleeping sickness, and, as arsenic was the body which appeared to have the greatest influence upon the disease, experiments were undertaken with this metal.

For some time after the commencement of his experiments, Ehrlich appears to have been of the opinion that the sole receptors between the chemical groups in the protoplasm of the parasites and the drugs used were the arsenic receptors. How Ehrlich could ever think that arsenic would find its chemical affinity in the protoplasm of cells, and, it is to be remarked, of only certain cells, is difficult to understand, and it is surprising that most of us should still consider that this element is of paramount importance in the synthetic anti-syphilitic remedies.

That other receptors existed as well, was shown only when it was noted that the action of arseno-phenyl-glycine was not affected by previously subjecting the parasites to the action of the arsenic derivative of phenyl-oxy-acetic acid. Then it was assumed that acetic acid receptors existed as well.

As fatty acid receptors were said to exist in the protoplasm of the parasites, it was only logical to suppose that amino-acid receptors would be found there also. Working upon this hypothesis, Ehrlich [1 2 3 4 5 6] discovered salvarsan.

Therefore, throughout his research, Ehrlich thought that the arsenic was the most important part of the molecule, and it is clear that it was only by going from A to Z, or by dint of exhaustive perseverance without any logical guidance, that he finally succeeded in finding a drug which had the desired therapeutic properties. All credit is due to Ehrlich, in that success was ultimately achieved, but one cannot help being struck at the terrible waste of time and effort, when several hundred bows were drawn at a venture before an arrow struck the gold. Even when light began to dawn, it was long shadowed by clouds of misconception.

According to Ehrlich,[5] salvarsan works by means of its arsenic receptors and its ortho-amino-phenol receptors. When it was discovered later that a certain drug had a fatal action on one kind of parasite and not upon another kind, although in both instances a combination occurred between the drug and the bodies of the parasites, some further explanation of the action of salvarsan was required. Consequently salvarsan was stated to act in the following way: The arsenic was considered to be the toxophore group, the benzene ring the carrier, and the amino-groups the haptophore groups. This is simply a transference of what is intelligible in simple language into what is unintelligible, though perhaps effective, owing to the mystery conveyed by the words in a coined language.

Summing up Ehrlich's views as to the mode of action of salvarsan, and expressing them in English, we find that a union takes place between the drug and certain parasites, with a destructive action upon the latter. What the nature of the union is, and why the death of only certain parasites should follow, the mere coinage of words does not explain.

There are two facts which stand out in Ehrlich's work on chemotherapy which appear to have been overlooked by him and his school, but which nevertheless are extremely important, as they go some way towards explaining the *modus operandi* of the subject. The first fact is, that trypanosomes can be rendered arsenic-fast, and that organisms which have been rendered arsenic-fast are immune to arsenical compounds only. To this there is an important exception. For instance, it was found that organisms which had been rendered immune to the arsenical compounds were also immune to pyronin.

The other fact is that, although salvarsan could be proved to enter into "combination" with the bodies of many, if not of all, parasites, *in vitro,* it did not necessarily exert a fatal action upon them all, but only upon organisms such as the trypanosome, the *Spirochæta pallida*, the spirillum of recurrent fever, etc.

From the points I have already brought forward dealing with the cause of syphilis, the chemistry of the organism, and the *rationale* of the Wassermann reaction, I will attempt to explain what is the probable action of salvarsan. When this is complete, I will deal with some points about the chemistry of the drug and its allied bodies, and then with the lines of research along which I thought chemotherapy would afford the best results, culminating in my discovery of some new drugs which are both better and less toxic than the arsenical compounds.

MODE OF ACTION OF ANTI-SYPHILITIC DRUGS.

Before discussing the mode of action of the anti-syphilitic drugs, it is necessary first to pay some attention to the way in which the body naturally protects itself against syphilis.

The protective power of the body can be divided into two groups: (1) the power which acts generally, *i.e.*, the lipoid-globulin molecules of the serum; (2) the power which acts locally, *i.e.*, the plasma cells.

The plasma cell is typical of chronic inflammation, whatever be the cause thereof. In all instances, the plasma cell is morphologically the same, but, although its gross action is similar in every instance, it is nevertheless specific in each case. To be more exact, one should call the specificity a group specificity, not an individual specificity, since the plasma cells behave in the same manner, as is to be here described, in trypanosomiasis as in syphilis, and also in some other diseases.

Take, for example, three plasmomata, one caused by syphilis, another by the organism of *Ulcus molle*, and the third by a foreign body. If an injection of any of the synthetic anti-syphilitic remedies be given to patients suffering from these three conditions, and sections be then made of all three lesions again, on examination it will be found that only the plasma cells in the case of syphilis have altered.

This specificity exhibited by the plasma cells is the same as that exhibited by the lipoid-globulin molecules of the serum, and, so far as it concerns chemotherapy, it is solely due to certain physical properties which these two protective powers possess in protozoal diseases. In other words, the specific action of the synthetic anti-syphilitic remedies is not due to the chemico-physical properties which constitute specificity, as we see it in infections and in immunity reactions.

Consideration must now be given to these peculiar physical properties of the anti-protozoal lipoid-globulin molecules.

In doing so, I will take the lipoid-globulin molecules in the serum first, as a summary of what has already been mentioned in the previous chapters will suffice to give the knowledge that is necessary here. The lipoid-globulin molecules in the serum of protozoal diseases, especially in syphilis, are characterised by their large size and their increased adsorptive capacity. As the disease becomes more and more chronic, the size of the molecules and their adsorptive capacity increase. As has been already explained, a parasite is vanquished *in vivo* by a change which takes place on its surface. This change causes a local alteration of the surface tension, which so disturbs the normal permeability that the organism perishes. This change is brought about by adsorption. Like most physical and chemico-physical actions, the phenomenon of adsorption is dependent upon active oxygen. Therefore, some ratio exists between the destructive power of a molecule and its supply of active oxygen. In the early stages of the disease, it is naturally to be expected that the supply of active oxygen is as great as the body, without extra assistance, can manage. If the supply of active oxygen is not sufficient to rid the host of his parasites, the disease becomes chronic, and, all the direct methods of producing active oxygen having failed, the host is obliged to obtain it indirectly. Active oxygen is obtained indirectly by increasing the reducing action, hence the reason why the lipoid-globulin molecules are larger, and have a greater adsorptive and reducing action in late than in early cases of syphilis.

There are three actions exhibited by the lipoid-globulin molecules in the serum: (1) adsorption; (2) oxidation; (3) reduction.

In virtue of the size and of the increased adsorptive capacity of the anti-protozoal lipoid-globulin molecules, it follows that their powers of oxidation and reduction can be enhanced by oxidising and reducing agents, and that a ratio must exist between the amount of active oxygen and active hydrogen produced and the degree of the adsorbed state in which the oxidising and reducing agents are.

More light can be thrown on this interesting subject, if attention be now paid to the active oxygen and active hydrogen contents of cells.

This may be best done by referring to the oxygen positions in the cells as a chain, "the oxygen chain," and then by referring to the hydrogen positions. Each link of the oxygen chain is made up of a peroxide and a ferment (peroxidase), which activates it in various degrees, according as to whether the first or last link of the chain is being dealt with. The first link is the red blood corpuscle, which contains a peroxide and a peroxidase; the ferment action is further increased by the iron in the hæmoglobin, which acts as a catalyst in the form of an hydroxide.

Oxidising enzymes have their action increased by metals; attention need only be drawn to the accelerating action manganese has upon some plant oxidases, in support of this statement.

Red blood corpuscles supply every tissue with oxygen in an active state.

The next link in the chain is a mast cell, one of the functions of which is to supply the basal cell layer of the epidermis with the active oxygen for the tyrosine-tyrosinase reaction, which results in the production of pigment, and this is one of the protective mechanisms of the body.

In support of this statement, reference need only be made to the well recognised increase of mast cells in *Urticaria pigmentosa*, ephelides, and all known pigmentary affections of the skin. Another function is, possibly, to supply another link with free active oxygen, namely, the nuclei of the cells of inflammation.

The next link in the chain is an eosinophile cell, the action of which is probably analogous to that of the mast cell. Between these two cells there is a fundamental difference, which is a difference of reaction. The mast cell granules are basophilic, the eosinophile granules are acidophilic. In some chronic infections, the mast cell predominates; in others, the eosinophile cell is most to the fore; both are carriers of peroxidases, hence the reaction of the base, as to whether it is acid or alkaline, possibly plays some *rôle* in the combating of infections.

The nuclei constitute the new link, as they contain a peroxide, and a ferment (peroxidase) for activating the same, but this ferment is not nearly so strong as the peroxidase in the first three links.

The activated oxygen in the nucleus is used mainly for the purposes of division, and partly by the protoplasm of the cell.

The last link in the chain is the protoplasmic, which contains a peroxide, but only a feeble peroxidase. The main peroxidase probably comes directly from the nucleus, and indirectly from other cells which contain peroxidases, through the blood serum.

The oxygen positions are those in which active oxygen can be demonstrated, the active oxygen being formed by the action of ferric hydroxide-protein (peroxidase) on hydrogen peroxide (peroxide), according to the following equations—

$$\mathrm{RFe} + 4\mathrm{H_2O} = \mathrm{R.Fe(OH)_3} + 4\mathrm{H} + \mathrm{OH}.$$

$$\mathrm{R.Fe(OH)_3} + 5\mathrm{H} + 2\mathrm{H_2O_2} = \mathrm{R.Fe} + 6\mathrm{H_2O} + \mathrm{O}''.$$

Wherever there are oxygen positions, there must also be hydrogen positions, and, speaking generally, those positions in which most oxygen can be demonstrated are those in which least hydrogen is observed, and *vice versâ*. The reducing action of mast cell granules is very marked, but not so that of eosinophile granules, while the reducing action of nuclei is *nil* except the protein covering the nucleolus. The reducing action of the protoplasm of the plasma cells is very marked, while its oxygen content is poor. Therefore, the hydrogen positions are to be found very marked in the mast cell granules, in the lipoid-globulin "envelope" of nucleoli, and in the protoplasm of plasma cells. These positions are recognised by the affinity they possess for pyronin and by the ease with which they decolourise methylene violet, methyl green, and malachite green.

The reducing action of the lipoid-globulin molecules, or, as it may be called, the reducase system, is analogous to the oxidising action of the oxidase system. A perhydridase takes the place of a peroxidase, and a perhydride the place of a peroxide. The action of perhydridase on a perhydride is to form active hydrogen, which corresponds to the active oxygen in the oxidase system.

The perhydridase is a di-sulphide-protein, and the equations representing its action are as follows:—

$$2R.SH + S = H_2S + R.S.S.R.$$

$$R.S.S.R + 2H + 4OH = R.S + R.SH + 2H_2O_2 + H^{\cdot}.$$

It is seen, then, that in the reducase system a non-metal takes the place of a metal.

Summing up the last few points, it may be noted that metals increase the production of active oxygen, and non-metals the production of active hydrogen. Therefore, from what has gone before, the reader will now see that extra active oxygen can be produced in the serum of the host, directly by the administration of strongly adsorbed metallic compounds and indirectly by the administration of strongly adsorbed non-metallic compounds. Without treatment, the lesions of syphilis vanish because the lipoid-globulin molecules of the serum and of the plasma cells contain a stereo-chemical molecular configuration homologous to those of the parasitic lipoid-globulin molecules, an homology which is necessary for perfect adsorption, and because they contain active oxygen necessary for the adsorption, which is formed directly by iron hydroxide and indirectly by a disulphide.

Since we do not know the chemical formula of the protozoal lipoid-globulin molecules, it is impossible to synthesise a compound homologous to them. Therefore, the only way in which we can assist the host is to increase his supply of active oxygen, which we do directly with arsenic, aluminium and iron and possibly also with antimony and mercury, and indirectly with iodine and sulphur.

Treatment then assists the host's resistance, by increasing his amount of active oxygen. Therefore treatment destroys the parasites indirectly, and not directly as Ehrlich thought.

The lipoid-globulin molecules circulating in the serum and in the plasma cells are the same. They constitute the antibody, and their specificity lies in their stereo-chemical molecular configuration. The action of antibody is one of adsorption, a phenomenon which can only take place in the presence of an oxidase-reducase system. The hydroxyl and hydrogen ions in the serum, plus this oxidase-reducase system, constitute what is commonly called complement. Therefore treatment stimulates the complementary action of the antibody, and it also increases the production of antibody. It is the antibody which destroys the parasite, the ferments are only the activators of its action, which is one of adsorption; consequently the ferments are not specific. When I first worked at this subject, and after I had learnt, from my investigations into the chemistry of the *Leucocytozoon syphilidis*, that the parasite was made up of a lecithin-globulin "envelope," which encased nuclein, and before I had discovered the *modus operandi* of the Wassermann reaction, which gave me the clue to the whole problem, I thought that the ferments themselves were specific, and I therefore attempted to find a specific lecithase and a specific protease. Failing in my attempts, and with the additional knowledge that I had gained in the meantime, I soon came to see that the ferments were the hydroxyl and the hydrogen ions, in the external phase (fluid part of serum), and the results of the action of ferric hydroxide-protein and di-sulphide-protein on these ions in the internal phase (solid part of serum). In support of this view, I have been able to collate the following facts:—

1. The granules in the epithelial cells of the choroid plexus give marked peroxidase reactions, and, as shown by Pighini[8], they are able to synthesise indophenol from a mixture of α-naphthol and di-methyl-phenylene-di-amine hydrochloride, the blue colour resulting being dependent upon the presence of active oxygen.

These granules also have a strong reducing action, shown by their forming a leuco-base with methyl green, due to the presence of active hydrogen.

2. These granules are increased in cases of degenerative encephalitis, which shows that the zymotic action is increased.

3. The cerebro-spinal fluid obtains its active properties from the cells of the choroid plexus.

4. I have been able to prove that the cerebro-spinal fluid from cases of degenerative encephalitis is rich in peroxidases (amino-acidases), while normal cerebro-spinal fluid contains none. The reducing action is likewise markedly increased.

5. The cerebro-spinal fluid, in cases of syphilis of the central nervous system, contains an excess of globulin.

6. This globulin is in an adsorption complex with "lipoids."

7. Peroxidases and perhydridases are carried by lipoid-proteins; therefore, not only is there proof that the cerebro-spinal fluid from cases of syphilis of the central nervous system contains peroxidases and perhydridases, but also that the ferments are carried by the lipoid-protein complexes, the physical action of which they accelerate.

This view is still further supported by the analogy to the blood serum and plasma cells.

Still more convincing is the fact, that I have been able to prove that the granules of the epithelial cells of the choroid plexus, and the tigroid or Nissl's granules to be found in the nerve cells, consist of lipoid-protein molecules, and behave to reagents similarly to the "envelope" of nucleoli, the protoplasm of plasma cells, and the "envelope" of the syphilitic organism.

Pighini[8] showed that Nissl's granules also synthesised indophenol, especially those around the nucleus, which was to be expected, since the nucleus contains a peroxide and a peroxidase. Nissl's granules also convert methyl green into its leuco-base, showing that they contain a perhydridase as well.

The explanation of the protective mechanism of the serum and of the cerebro-spinal fluid against the spyhilitic parasite, by means of adsorption, a process which is accelerated by such ferments as a peroxidase and a perhydridase, is simple; in fact, it is its simplicity which makes one think that it is correct, for the more one considers how the body protects itself, the more convinced one becomes that it is not nearly so elaborate a process as all have been led to believe.

After all, why should such an exceedingly complex ferment as a lecithase or protease be manufactured specifically against syphilis? Supposing another protozoal disease sprang up in our midst to-morrow, the body would be ready to protect itself, and it could not possibly, in the short time at its disposal, manufacture highly complex specific ferments.

A most interesting point now crops up, namely, why are the spirochætæ destroyed more quickly than the other phases, and why so quickly by the metallic compounds? Chemistry showed that the male gamete, or *Spirochæta pallida*, had the strongest reducing action of all the phases. In *in vivo* staining, it showed a marked affinity for methylene red, and it increased the reducing action of the female cell after impregnation.

In this very strong reducing action, in my view, lies the solution of the problem as to why the adult male phase, the female phase after impregnation, and not the other phases, stain with silver nitrate in Levaditi's method of staining, and as to why metallic compounds act more markedly upon the first two than do the non-metallic compounds.

The reducing action is due to an unsaturated fatty acid—a substance in which the male cell is especially rich, for two reasons: (1) because it is the result of an intracellular development; (2) because it has a very important function to perform, namely, that of impregnation. In other phases, where the metabolism is less active, and the cells are more or less in resting forms, the fatty acids are more likely to be saturated than unsaturated.

The more unsaturated a fatty acid is, in a complex, the more free hydroxyl-groups will there be,

To these free hydroxyl-groups different chemical substances can become attached, especially if they are free metals possessing a positive charge; therefore the *Spirochæta pallida*, owing to the fact that it contains more free hydroxyl-groups, can have its lipoid-globulin molecules altered by substances which combine immediately with them.

In staining tissue with silver nitrate, in order to get a black colour, two things are necessary: one is that the silver must be adsorbed; the other is that it must be reduced *in situ*. Owing to the free hydroxyl-groups in the lipoid-globulin molecules of the *Spirochæta pallida*, the silver is readily taken up and reduced by the pyrogallic acid. In the other phases, on the other hand, there are no free hydroxyl-groups to take up the silver, so they therefore cannot stain black. The action of the metallic compounds is also to be explained in this way.

The arsenic, aluminium, and iron, in their respective compounds, salvarsan, aluvine, and ferrivine, become liberated after the amino-groups in the compounds have become adsorbed by the lipoid-globulin molecules. The free metals, being positively charged and tri-valent, will adsorb three atoms of the negatively charged hydroxyl ion, and, as more of the metals become deposited in those positions in which the parasites are located, in their avidity for free hydroxyl ions they will naturally seize upon some belonging to the adult male phase and to the female phase after impregnation, hence the immediate death of these two phases.

As there are no free hydroxyl-groups in the other phases, the metals cannot become so readily attached to them. The destruction of the other phases is brought about, indirectly, by the stimulating effect the metals have upon the adsorptive action of the protoplasm of the plasma cells, and the lipoid-globulin molecules (antibody) of the serum. Therefore, the action of salvarsan upon the spirochætæ is wholly a direct one, and upon the other phases an indirect one.

The question might now very naturally be asked, If the action of salvarsan is an indirect one, why should arsenic be more active than mercury, since under ordinary circumstances the latter is a more powerful anti-syphilitic remedy?

In my opinion arsenic acts as a catalyst, *i.e.*, it accelerates a reaction going on spontaneously, but more slowly, without its assistance.

A ratio exists between the intensity of action of a catalyst and the degree of the colloid state in which the catalyst is. In salvarsan the arsenic is in a colloidal state, hence its action would necessarily be greater than that of any mercurial compound which we are in the habit of using. The proof of what has been said can be found, if we compare the action that different manganese compounds have on plant oxidases. Manganese formate, for instance, has nothing like such a powerful accelerating action upon plant oxidases as manganese hydroxide. The latter is colloidal, and the former is not.

In many other synthetic organic arsenical compounds, the arsenic is also in a colloidal state, but the action of the drug is very inferior to that of salvarsan. Therefore this fact at once suggests that salvarsan when injected becomes fixed to the substance which the arsenic is going to accelerate, and it proves that a ratio exists between the therapeutic action of a drug and the degree of its adsorbed state.

The proof that this is not pure theory is seen in the following statements: I showed in my work on the Wassermann reaction that complement and antibody were contained in the one molecule in the presence of water, and that they constituted the lipoid-globulin particles or the protective substance of the serum. That the action of antibody was to adsorb its antigen, which it did by means of its ferment properties which constitute the complement, and that adsorption resulted in precipitation and final hydrolysis of the bodies adsorbed. Further, that the action of "606" was to break down the lipoid-globulin molecules of the serum and of the plasma cells. Finally, that adsorption took place between the respective amino-groups.

Now, salvarsan has amino-groups which other organic arsenical compounds have not, hence sufficient proof can be brought forward to allow the assumption to be made that salvarsan fixes on to the lipoid-globulin molecules of a syphilitic serum and of the plasma cells, in a case of syphilis, by nature of its amino-groups, hence allowing the arsenic free play as a catalyst. Since the "606" molecule enters into adsorption with the lipoid-globulin molecules of a syphilitic serum and of the plasma cells in a case of syphilis, it will be seen that Ehrlich's statement that the drug is parasito-tropic only, and not organo-tropic, cannot hold good. For a drug to be parasitotropic it must be organo-tropic; indeed, its organo-tropic properties are far more important than its parasito-tropic properties.

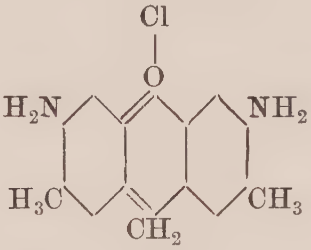
It is possible that amphoterism plays a part in the way in which salvarsan acts. I have already shown that its action is greatest on the spirochætal phase, because the spirochæta is the most basophilic of all the phases, owing to the fact that it contains an unsaturated fatty-acid-group, hence it would be expected that the action of "606" was more marked in the late stages of syphilis than in the early ones. This happens to be the case, since the lipoid-globulin molecules of the serum are more readily broken down by "606" in the case of a late recurrent syphilide than in the case of generalised syphilis. I have pointed out that serum from a late case of syphilis is richer in carboxyl-groups than from a case of early syphilis.

As the former serum is more readily broken down by salvarsan, and as the spirochætal phase is the phase most easily destroyed, it is reasonable to assume that there is a ready attachment between salvarsan and the carboxyl-groups of the bodies aforementioned.

Amino-groups can be fixed by carboxyl-groups, therefore one cannot say at this stage whether the attachment just mentioned is dependent more upon the hydroxyl-groups or upon the amino-groups of the "606." With this matter I will deal further on. Suffice it to say, at present, that the amino-groups are the most important in the salvarsan molecule. That this is true, is seen from the fact that any alteration in the amino-groups diminishes the efficacy of the drug. Hence the reason why neo-salvarsan is not so potent as salvarsan, in spite of the fact that it contains two hydroxyl-groups, which are less stable than the ONa groups in salvarsan. The reason why adsorption appears to be more marked when one of the bodies adsorbed is rich in carboxyl-groups is due to the fact that these groups increase the permeability of that body, and therefore render easier the attachment between the respective amino-groups.

The importance of the two points which were brought out in Ehrlich's work can now be discussed. If there is a relationship between arsenical immunity and pyronin immunity, it cannot be the arsenic that the parasites become immune to.

If the formula of pyronin is studied—



Pyronin.

it will be noticed that there are two amino-groups. Note further that the amino-groups are in the ortho-position, as they are in salvarsan. Now there are two amino-groups in salvarsan, and from what has been already said it would appear to be due to these two amino-groups that the drug becomes attached to the syphilitic parasites, the plasma cells, and the lipoid-globulin molecules of a syphilitic serum.

I have little doubt in my own mind, but that immunity is mainly dependent upon the adsorption between certain amino-groups, and the case in point certainly favours such a view.

In view of the fact that parasites which are salvarsan-fast are also pyronin-fast, it proves that immunity is not acquired against the arsenic, therefore the parasites cannot be strictly said to be arsenic-fast. So far as future experimental work is concerned, there is a very important fact to have in front of one, namely, that an immunity cannot readily be produced against a catalyst. The fact that salvarsan can become attached to parasites, and yet not be fatal to them, is an observation in which there is a great deal wrapped up. The reader must observe that such an adsorption could only have been proved by experiments conducted *in vitro.*

During the time when I was working at the *rationale* of the Wassermann reaction, I thought that for adsorption to take place between two molecules, both of which possessed amino-groups, it was necessary that they should have an homologous stereo-chemical molecular configuration. This idea soon proved to be wrong, when fixation occurred between both a non-specific antigen, and an antigen whose amino-groups had been converted into methylene-imino-groups by formalin, and the reagin or serum lipoid-globulin molecules from a case of syphilis.

As a result of further experiments, I proved[10] that the complement-fixation test, as applied to syphilis, in contradistinction to the true bacterial complement-fixation tests, depended not upon the molecules having an homologous stereo-chemical molecular configuration, but solely upon their number and size.

Application of this to the action of salvarsan and the other synthetic compounds, confirms the points just mentioned. The attachment of these compounds to the syphilitic parasites, to the plasma cells, and to the lipoid-globulin molecules of the serum in a case of syphilis, cannot possibly be due to an homologous stereo-chemical molecular configuration, between the adsorbed molecules, since, for one thing alone, the synthetic compounds are optically inactive bodies.

The adsorptive capacity, as a whole, appears to be greater in the case of a syphilitic serum than in the case of a serum from any bacterial disease, and it appears to be greater the later the case of syphilis.

As the lipoid-globulin molecules from a late case of syphilis are richer in carboxyl-groups than those from an early case of syphilis, it follows that any substance they happen to adsorb will tend to break down the molecules in virtue of the increased permeability produced by such groups. The first action of breaking down the lipoid-globulin molecules is to increase the area over which they can work, hence the reason why late syphilitic lesions disappear more quickly under treatment than do early ones. In the case of syphilis, and in all protozoal diseases, the lipoid-globulin molecules are bigger, and have a greater adsorptive capacity than the lipoid-globulin molecules in the serum of bacterial diseases.

The bigger the size of the molecules, and the greater their adsorptive capacity, the more easily will a drug like salvarsan be adsorbed, and the more readily will the adsorbed compound break down. When the lipoid-globulin molecules first break down, the area of their action is widened, hence the catalytic action of the arsenic will have its fullest play. If salvarsan becomes attached to small molecules, the adsorbed compound does not break down; at all events, not until much later than it does in the case of syphilis. Moreover, so much of the arsenic compound is not adsorbed. This means that the arsenic can get little chance of acting as a catalyst. Therefore, salvarsan acts in protozoal diseases because of the physical state of the lipoid-globulin molecules of the serum, and it fails to act as a bactericidal agent because the lipoid-globulin molecules in the serum of bacterial diseases do not possess the requisite physical properties. From the fact that Ehrlich noticed it was only such organisms as the *Spirochæta pallida*, the spirillum of recurrent fever, and the trypanosome, which were killed by the action of salvarsan *in vitro*, two important conclusions can be made: (1) that the spirillum of recurrent fever and the trypanosome, are killed because of the free hydroxyl-groups their molecules contain; (2) that the trypanosome is not the sole cause of sleeping sickness, as salvarsan no more cures trypanosomiasis than it does syphilis.

To sum up the points which stand out, we can in short say that strongly adsorbed metallic and non-metallic compounds become attached to the lipoid-globulin molecules of the syphilitic parasites, the plasma cells, and the serum, by virtue of their amino-groups and of the peculiar physical properties of the protozoal lipoid-globulin molecules. The metallic compounds attack those phases of the *Leucocytozoon syphilidis* in which reaction is most marked, especially the spirochætal phase, in virtue of its free hydroxyl-groups.

Both metals and non-metals act as catalysts, that is to say, they accelerate the complementary or oxidase-reducase action of the lipoid-globulin molecules of the serum and the plasma cells, by increasing directly and indirectly the supply of active oxygen, which is necessary for the action of adsorption—a chemico-physical action which destroys the parasites.

Chemistry of the Arsenic Compounds.

Magnificent and admirable though Ehrlich's work was, there is one aspect of it which does not call for admiration. I refer to the fact that the root-idea of "606" was due to a Frenchman, Béchamp,[11] and to the fact that the formaldehyde sulphoxalate process, by which "914" was prepared, was first practised at the dye works of Zündel, in Moscow. No recognition is given to either of these sources of inspiration. It is true that Ehrlich and Bertheim[1] corrected the orientation of Béchamp's compound, but that is a small matter compared with the synthesis of the body. Further, I throw out the suggestion to chemists, is Béchamp's orientation really wrong?

It is also to be regretted that Ehrlich allowed his work to be patented, and to be patented in a way which, it was hoped, would prevent others from continuing or improving upon it.

Fortunately, there is hardly a statement of Ehrlich's which now holds good, and I am of the opinion that, before very long, not one of the arsenical compounds will remain in general use.

While on the subject of ethics, so far as they affect chemotherapy, it would be as well to trace the development of the substitution products for salvarsan and neo-salvarsan since the European War commenced.[12] The Board of Trade handed over the patent rights to an English and to a French firm, with the proviso that these firms should submit the samples prepared to biological tests before they were sold. The National Medical Research Committee, which had the testing of the drugs in hand, sent two communications[19 20] to the medical press, to the effect that the English and French substitutes for salvarsan and neo-salvarsan were chemically identical with, and just as good as, any of the German products previously made.

Because the substitution products have the same percentage compositions as those given for the German drugs, it does not follow that the two are chemically the same. In my own experiments with the sulphur and metallic compounds, I have been able to prepare some, the compositions of which were identical, but which were not only different to look at, but their therapeutic action was different. The difference is due to the varied methods of preparation. For two like substances to be absolutely chemically the same, it is necessary that they should be prepared in exactly the same way. It is practically certain that no one outside Hoechst knows how salvarsan and neo-salvarsan are prepared. Furthermore, treatment after preparation is an item which most seem to ignore, and, when colloidal compounds are concerned, a trace of impurity may be sufficient to prevent the onset of symptoms which may occur when the absolutely pure compound is used.

Salvarsan and neo-salvarsan are closely related to dyestuffs, and are made by Meister, Lucius, and Brüning, who are dye manufacturers. Dyestuffs are often blended, just as teas are blended, and this blending is one of the most valuable secrets of the industry. The so-called synthetic indigo-blue, which is used so largely for dyeing textiles, is, I believe, a blend of 15 dyes.

In some of my experimental work on colloidal compounds, I found that a trace of egg-white, gelatin, or agar was sufficient to prevent death, which invariably followed the administration of the pure substance.

The mild cases of anaphylactic shock, which sometimes occur after the substitution products, can be prevented, if the patient's blood is allowed to mix with the "solution" first.

Clinical experience soon showed that the substitution products were not as good as the German products, and that they were dangerous to use. Soon after neo-kharsivan—the substitute for neo-salvarsan—was put upon the market, owing to its highly toxic nature, its supply was stopped, and we have been without an actual substitute for neo-salvarsan since.

It is futile to rely upon biological tests to tell whether a drug is safe for wholesale use on human beings. Biological tests are of value only in so far as they give an indication as to whether it is safe to try the drug on a human being. To give an example. Optic atrophy can be produced in cats with doses of the arsenical compounds, which produce no toxic symptoms in dogs. If Ehrlich had studied the human body, and had not drawn all his conclusions from his animal experiments, and from what he noticed in test tubes, he would never have made the mistakes he did make. Even Ehrlich, who was not a clinician, went so far as to get clinicians to use salvarsan and neo-salvarsan on a few selected cases, before they were allowed to be used on a large scale.

It seems to me a pity that the Board of Trade should have handed over the preparation of the substitute for salvarsan to a commercial firm, and not to one or more medical men. If it had been handed over to medical men, and if success had come to their efforts, it would have raised medical prestige enormously in this country.

To my mind, the soundest plan to have adopted would have been, first of all, to unravel the mysteries of chemotherapy, and to have discovered why salvarsan and neo-salvarsan have such a marked anti-syphilitic action, and then to have set to work to prepare other drugs having a similar action, the manufacture of which would not have infringed the German patents. If success had crowned these efforts, there would be no apprehension as to what would happen in future.

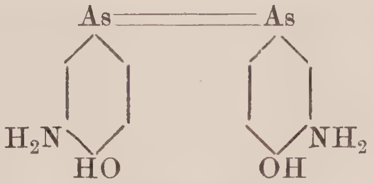
Summing up the matter, I must say that, in my opinion, neither the English nor the French substitution products for salvarsan and neo-salvarsan are as safe for general use as they might be.*

Other workers in France, in Canada and elsewhere have likewise fallen victims to the German idea that arsenic is essential, and have spent their time in preparing drugs containing this toxic metal. According to certain reports a drug called margol, which has three elements in its molecule—arsenic, antimony, and silver—has been prepared in France. Margol has been given the number of 102, and it is alleged to be twice as powerful as Ehrlich's "606," and quite harmless. How true these allegations are likely to be, the following pages will show.

---

* Furthermore, imitation does no credit to British medicine, not even when it is perfect.

Salvarsan.—One of the most striking points, to my mind, about salvarsan, and one which shows clearly how much Ehrlich was in the dark, is the fact that the compound is neutral.

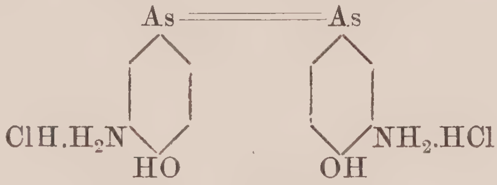


Salvarsan.

Note that the two basic amino-groups are neutralised by the two hydroxyl-groups, showing that Ehrlich intended to have as many arrows as possible in his quiver. I should rather fancy that the wish was father of the thought, since the hydroxyl-groups had to be put in to render the body soluble later.

Salvarsan is a body which is both acidic and basic, or amphoteric, and it also contains an element which is both metallic and non-metallic, and one which has a varying valency.

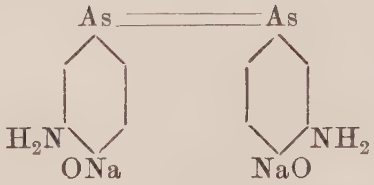
The body is insoluble in water, and on this account the hydrochloride was prepared. Its formula is



Salvarsan hydrochloride.

The hydrochloride dissolves sparingly in water, but its solution is strongly acid, hence it is not very suitable for intravenous administration. It may be injected intravenously, and is, on the whole, more potent than the basic solution prepared from it, but it is at the same time more toxic.

From the hydrochloride the sodium salt is prepared by adding sodium hydrate to the acid solution, and this renders it more soluble in water. Therefore the drug is generally administered in this form—



The sodium salt of salvarsan.

Comparing this formula with that of the fundamental substance, it will be seen that the two hydroxyl-groups (OH) have been replaced by ONa groups. The hydroxyl-groups were acidic; the ONa groups are basic, as are also the amino-groups (NH_2), so that the compound injected is distinctly basic.

A nice point now arises. When the compound is injected, does it remain basic or does it become amphoteric? In other words, are the ONa groups replaced by hydroxyl-groups? Knowing that the blood tends to maintain a definite standard of alkalinity because of the excess of hydroxyl ions, it is practically certain that such a change does occur. We know, too, that if this standard be disturbed, the blood will proceed, with almost inconceivable rapidity, to re-establish its standard. Now, therefore, if an alkaline compound be injected into the body, the blood will at once reduce the alkalinity of that compound. In the case of the sodium compound which we are considering, this can only be done by converting the ONa groups into hydroxyl-groups, with the formation of neutral sodium salts. That is to say, an alkaline ONa group is converted into an acid hydroxyl-group, with the formation of a neutral sodium salt, so that the final result is a reduction in the total alkalinity of the compound injected.

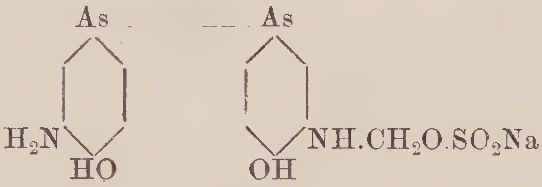
To what does this lead? To the highly important fact that now we again have the original base-substance, which is amphoteric, by reason of its possession of basic amino-groups and acidic hydroxyl-groups. The importance of this fact consists in the point that such an amphoteric compound will be ready to attack either acidic or basic compounds. Reference to my work on the chemistry of the *Leucocytozoon syphilidis* will show proof of the fact that some phases in the life-cycle of that organism are more basophilic, and that some are more acidophilic than others. It is therefore theoretically possible that reaction between these phases and the amphoteric salvarsan plays a *rôle* in the action of the drug upon the syphilitic organism. The *rôle* played by reaction must be quite insignificant, since any change produced is wrought before the compound reaches the organism, and because the action of the compound on the parasite is an indirect one.

It may be objected to this line of argument, that the only active principle in salvarsan is the arsenic, and that the other facts are irrelevant, and this is a view which is very largely held.

Let us consider the action of the arsenic. In the first place, the arsenic will exert no action until it has been freed from the compound. An amphoteric compound will tend to break down when it comes in contact with either a basic or an acidic substance. Therefore the arsenic will tend to be set free for action in contact with the *Leucocytozoon syphilidis*, the plasma cells, and the lipoid-globulin molecules of the serum. It is, therefore, fair to conclude that salvarsan is particularly well fitted to apply its arsenic at the most necessary point.

In the second place, consider the action of the arsenic. The arsenic is excreted unchanged. That is to say, it is tri-valent when injected, and it is also tri-valent when excreted. To what conclusion does this lead us? To the conclusion that the arsenic acts as a catalytic agent. In what way can the arsenic act as a catalyst? In all probability it acts as a producer of active oxygen—in other words, as a peroxidase.

Neo-Salvarsan.—Neo-salvarsan is the commercial name for sodium-di-oxy-di-amino-arseno-benzene-mono-methane-sulphonate, and its formula is—

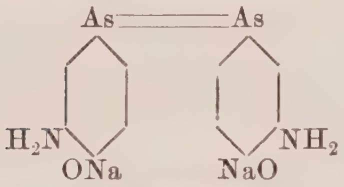


Neo-salvarsan.

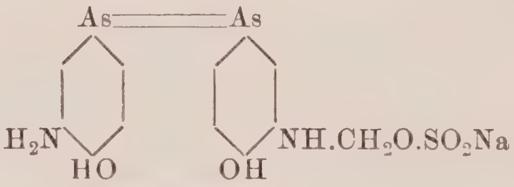
Salvarsan was the 606th body investigated, and neo-salvarsan was the 914th. It is obtained by the action of formaldehyde sulphoxylate upon "606." It is rather surprising that 307 preparations were made before neo-salvarsan was tried, for it is well known that the addition of a sulphoxylate group is a great aid in preparing a soluble body. The base di-oxy-di-amino-arseno-benzene was the 592nd preparation tried—salvarsan is the hydrochloride of this base, and it was the 606th preparation tried. Again, it is a matter for some surprise that 13 preparations were made before the hydrochloride was investigated. The marked advantage possessed by "914" over "606" is the greater solubility of the former. When a body is to be injected in solution, the advantage of ready solubility is obvious. Both these bodies have a great affinity for oxygen, and they must therefore be kept in an inert gas.

Now let us consider the therapeutic action of these two bodies, and let us see if the difference in action can be explained by their differences in chemical constitution.

The difference in action of these two drugs is that "914" is less potent than "606," though the *dosis tolerata* of the former is greater than that of the latter. The actual weight of arsenic injected is greater in the case of "914" than in that of "606." In the case of salvarsan the arsenic content per cent. is 31·56, while in neo-salvarsan it is 32·10. We therefore must conclude that arsenic is not the sole factor in the action of the drugs.



Salvarsan.



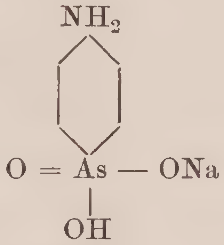
Neo-salvarsan.

The above formulæ represent the composition of the compounds as injected. The arsenic has been already considered.

"606" contains two basic ONa groups, and "914" contains two acidic hydroxyl-groups. "606" contains two basic amino-groups; "914" contains one amino-group and one amino-formaldehyde-sulphoxylate-group. The latter is a very important point, for an amino-group very readily attaches to it other complicated groups, whereas the ONa groups are rather stable. The fact that one of the amino-groups in "914" is already attached to a complicated group destroys its usefulness. We are, therefore, led to the conclusion that a most important factor in these two compounds is the amino-group. Further evidence in support of this conclusion is given by the action of arseno-phenyl-glycine, which will be referred to later. In this compound there are two amino-acetic acid-groups, and the therapeutic action of the compound compares unfavourably with that of "606" and of "914."

Earlier Arsenical Compounds.—Before salvarsan came into use, three other ring carbon compounds of arsenic were widely used.

(1) Atoxyl, or mono-sodium-para-amino-phenyl-arsenate. The composition of this body is shown in the formula—



Atoxyl.

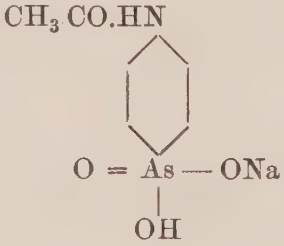
Compare this formula with that of salvarsan, and it will be seen that there are several points of difference:—

1. (1) The compound contains only one benzene ring instead of two.
2. (2) The arsenic is penta-valent instead of tri-valent.
3. (3) The hydroxyl-group is attached to the arsenic atom and not to the benzene ring.
4. (4) The arsenic is in the para-position to the amino-group and not in the meta-position.

This body is amphoteric, for the hydroxyl-group is acidic, and the ONa and amino-groups are basic.

Atoxyl is unstable, and it exerts a highly toxic action on the optic nerve, hence it was rapidly superseded by—

(2) Arsacetin, which is the acetyl compound of the above body. The formula of arsacetin is—

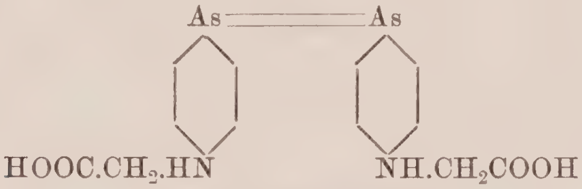


Arsacetin.

This body is less toxic and more stable than atoxyl. The addition of an acetyl-group ($CH_3.CO$) is a common device in chemistry when one desires to render a body more stable.

Both of these bodies are much more toxic than salvarsan, and they are so because the arsenic is penta-valent. The fact that the acetyl compound is the less toxic, leads to the conclusion that the arsenic does not exert its full toxic action until the compound is broken down. Now, in order that any compound may exert an action on the syphilitic organism, the compound must break down, therefore we must conclude that arsenic should be tri-valent in these therapeutic agents. This brings us to—

(3) Arseno-phenyl-glycine, which has the formula—



Arseno-phenyl-glycine.

Let us note the characteristic points of this compound.

(1) It has two benzene rings.
(2) The arsenic is tri-valent.
(3) It is a substitution product of acetic acid.
(4) The arsenic is in the para-position to the nitrogen.
(5) It contains two acidic hydrogen atoms, the hydrogen atoms in the carboxyl-groups (COOH).
(6) It contains two basic hydrogen atoms, the hydrogen atoms attached to the nitrogen atoms.

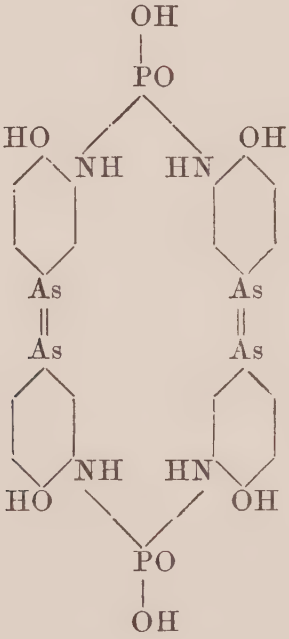
From the last two points it follows that the body is amphoteric, but the basic nature of the body is very feeble, for not only is one basic hydrogen of each amino-group substituted, but also an acidic carboxyl-group is attached to the substitution body.

The advantages which this body possesses over atoxyl are that the arsenic is tri-valent and that there are two benzene rings.

The disadvantage is that the basic nature of the body is, so to speak, destroyed by the two carboxyl-groups. It is, of course, possible that the acetic acid-group is broken off in the blood stream, for the blood is faintly alkaline. This would leave a basic amino-group, and the arsenic compound would cease to be amphoteric and would be basic only. Judging the action of these drugs by clinical methods, it would appear that the action is regulated by the constitutional formula of the drug before it is injected, and that, however near to the base a salt may be converted by the serum, its action is never so powerful as when a similar compound to the converted product is itself injected.

French Arsenical Compounds.—Several other arsenic compounds have been synthesised since salvarsan and neo-salvarsan have come into wide use. Mouneyrat has prepared two such bodies—"galyl" and "ludyl"—and he considers that they have some advantages over the earlier preparations.

Galyl is the commercial name for tetra-oxy-tetra-amino-tetra-phenyl-arseno-di-phosphoric acid, and its constitutional formula is :—



Galyl.

The chief characteristics of this body are that it contains six acidic hydroxyl-groups and four tri-valent arsenic atoms. The four amino-groups (NH) are linked up with acidic phospho-groups, and so they have lost their basic nature.

The three elements—arsenic, phosphorus, and nitrogen—are closely related in chemical characteristics. One would therefore naturally expect that the introduction of phosphorus would increase the activity of compounds such as we are considering. It must, however, be noted that the phosphorus here introduced is in an acidic group, and so is in no way comparable with the arsenic or nitrogen. Galyl is dissolved in sodium carbonate solution before injection, and so it is rendered basic. I have had considerable experience with this drug, and my results lead me to the conclusion that it is not quite so therapeutically active as salvarsan, and somewhat more toxic, but nevertheless it is the best arsenical compound now obtainable. This drug shows still further that the arsenic is not the only important group in the compound, since it contains 35·3 per cent. arsenic.

If salvarsan is compared with atoxyl and arsacetin, it will be seen that in salvarsan the arsenic is tri-valent, while in atoxyl and arsacetin the arsenic is penta-valent. Arsenic in a penta-valent state is less active in exerting its therapeutic influence on protozoa than when it is in a tri-valent state. This is due to the fact that, in a molecule in which all the valencies are satisfied, the influence desired is less likely to act than when some of the valencies are not satisfied.

In this connection Hardy's[13] rule is an important one to bear in mind, viz., that the precipitating power of an electrolyte depends upon the valency of the ion. From this it is clear that no compound of a uni-valent ion would be likely to have a therapeutic action at all akin to that of salvarsan. Therefore, so far as future research is concerned, the element must at least be a di- or, better, a tri-valent ion.

Apart from mere valency, two other important considerations present themselves: (1) the degree of adsorption of the compound; (2) the atomic weight of the element.

Ishizaka[14] made the important observation that a strongly adsorbed organic anion, such as salicylic acid, was more powerful in precipitating aluminium hydroxide than a weakly adsorbed uni-valent inorganic ion. Hence, to obtain the best therapeutic action with a compound, we see that it must be organic, and the more strongly adsorbed it is the better, the reason being that a ratio exists between the strength of adsorption of the compound and the power it has in diminishing the mechanical surface energy. It is now clear why salvarsan should be so much more effective than arseno-phenyl-glycine, arsacetin, or atoxyl. Freundlich and Schucht[15] found that, in the precipitation of a negative colloid by kations, those of the heavy metals and of organic bases were more active than would be expected from their valency, and that this was to be accounted for by the fact of their great mechanical adsorption. This observation shows us, then, not only why mercury should be useful in syphilis, but also why it should be toxic. Owing to the great mechanical adsorption exerted by the heavy metals, it is at once obvious that strongly adsorbed organic compounds of them would be too toxic for injection.

Studying the *rationale* of chemotherapy in this way, one may be saved from doing an amount of useless experimental work by excluding all uni-valent and di-valent elements, and those tri-valent elements which have high atomic weights.

Seeing that arsenic, a tri-valent element in the form of a di-oxy-di-amino-benzene compound, may be toxic, the atomic weight being only 75, and that the corresponding compound of antimony, di-oxy-di-amino-stibino-benzene, is more toxic still, the difference in the atomic weight being only $(120-75=)\ 45$, it is perfectly clear that, if we wish to find a non-toxic compound, we shall have to seek for elements which are tri-valent with a smaller atomic weight. Before mentioning the elements I tried there are one or two other points to be considered.

Di-oxy-di-amino-stibino-benzene, according to Hügel,[16] is not only more toxic than its corresponding arsenic compound, but its therapeutic action is infinitely less. Comparing the two bodies, we find that the arsenic in salvarsan is both metallic and non-metallic, while in the antimony compound just mentioned the antimony is entirely metallic. It therefore struck me that a less metallic element than arsenic might prove to be its therapeutic superior, in any case the probability was great that it would be considerably less toxic.

Salvarsan is a strong oxidising body, indeed its arsenic acts catalytically as a peroxidase, but at the same time it is a reducing agent, although its properties in this direction are probably not made use of in the body. This led me to think that a similar body to salvarsan, which had stronger reducing properties, would probably do just what salvarsan failed to do.

I wanted, then, an element which had the following properties: (1) of more than one valency; (2) an atomic weight of less than 75; (3) a non-metal; (4) a reducing agent.

Only two elements occurred to me which would fulfil these conditions, and they were phosphorus and sulphur. I discarded phosphorus, owing to the fact that it can produce very toxic symptoms, such as fatty degeneration of the liver, and I made experiments with sulphur and succeeded in making a compound which has in some respects a more powerful therapeutic action than that of salvarsan. I prepared several compounds, but soon found that one was much superior to the others, and this one was di-ortho-amino-thio-benzene or, as it is commercially called, "intramine."

Further research showed me that it was highly probable that other metallic compounds could be found, which would prove to be as efficacious and less toxic than salvarsan. Seeing that the metal must be tri-valent and not of high atomic weight, I began to experiment with iron, manganese and aluminium. In a short time I was able to prepare some compounds of iron and aluminium, which turned out to be non-toxic and to have, if used in sufficient and repeated doses, a marked therapeutic action like salvarsan.

Salvarsan, aluvine, and ferrivine (the commercial names given to the aluminium and iron compounds) are, as we have already observed, oxidising agents, while intramine is a reducing agent. In other words, the former increase the production of active oxygen which is necessary for adsorption to come into play, while the latter accelerates the splitting up of water, which frees the peroxides, to become later activated by the peroxidases to which metals are attached. Therefore it might be supposed that the one would enhance the value of the other; clinical experience has fully justified the supposition.

In the early stages of syphilis oxidation is more marked than reduction, and in the late stages *vice versâ*. Therefore the metallic compounds would be expected to act more quickly in the early stages of syphilis, and the non-metallic compounds more rapidly in the late, exactly what clinical experience has proved.

In closing this part of my subject I must briefly refer to two new salvarsan compounds prepared at Ehrlich's suggestion, as they show how very empirical the German view of chemotherapy is. One compound contains copper in addition to the arsenic, and the other contains sodium. Copper is a di-valent metal, and hence must rob the tri-valent arsenic of some of its action, as the insertion of copper in the compound has to be in part at the expense of the arsenic. The insertion of sodium must render the compound still less therapeutically active, as sodium is a uni-valent element. Therefore, we may safely conclude that Ehrlich, even up to the time of his death, was entirely ignorant of the principles which govern chemotherapy, and of the action of the compounds prepared.

The Analogy of Immunity to Chemotherapy.

In the complement-fixation test, we find two distinct reactions, one specific, the other not. The specific one is dependent upon the amino-groups in the antigen having a stereo-chemical molecular configuration homologous to those of the antibody. The non-specific test is due to the size and increased adsorptive power of the resisting substance.

Apply this to chemotherapy, and it will be seen that the action of the drugs we use in syphilis is exactly similar to the non-specific test.

A syphilitic serum will, owing to the size and increased adsorptive action of its particles, adsorb any other colloid, and, in the presence of complement, the two adsorbed bodies will be precipitated. The colloid adsorbed by the syphilitic serum can be either an emulsion or a suspension colloid, but the adsorption is more pronounced with the latter than with the former. A non-syphilitic serum will adsorb both an emulsion and a suspension colloid, but, in the presence of complement, precipitation will not occur with the former, since the adsorption is not sufficiently marked to destroy the complement.

Comparing what we have observed *in vitro* with what presumably takes place *in vivo,* will carry us another step further.

The particles both in a normal serum and in a syphilitic serum will adsorb suspension colloids, hence, why not cure all protozoal diseases by intravenous injections of suspension colloids?

Unfortunately, it is impossible, since minute intravenous injections of high dilutions of suspension colloids cause almost instantaneous death. Even if it was possible, metallic suspension colloids would have only a small therapeutic action, because the elements are not in a powerfully adsorbed state. This would also apply to non-metallic elements, such as sulphur and phosphorus, but not to iodine, as it does not form a protein compound. The reason why the adsorption with a suspension colloid appears to be more marked than when an emulsion colloid is used, is simply because *in vitro* the electrolytic dissociation of the ions cannot be rectified, as it can be *in vivo.* Therefore, the adsorption just referred to is more apparent than real.

The particles both in a normal serum and in a syphilitic serum will adsorb emulsion colloids, but unless the emulsion colloid injected contains amino-groups which have the same stereo-chemical molecular configuration as the amino-groups of the lipoid-protein particles of the serum, the adsorption will not be sufficiently pronounced, in the case of the normal serum, to produce any alteration of surface tension, or any change in the permeability of the bodies adsorbed. Only in the case of the syphilitic serum will the adsorption be sufficiently pronounced to produce the physical changes mentioned. This being the case, it is to be expected that an intravenous injection of any emulsion colloid will have a therapeutic action in syphilis. The expectation was fully realised. In several cases of syphilis, I gave intravenous injections of aluminium hydroxide and ferric hydroxide, both suspension colloids, which I had converted into emulsion colloids by gelatin, glycine, and albumin, with the result that not only did the symptoms fade, but both these metallic emulsion colloids enhanced the therapeutic action of intramine.

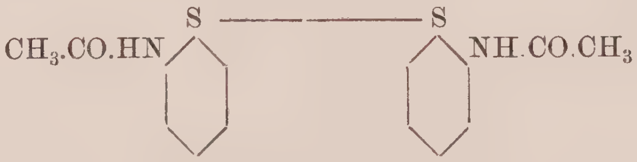
The problem which now naturally presents itself is, Are certain emulsion colloids more active than others?

In the Wassermann reaction, the antigen which acts best, that is to say, does not give positive reactions with normal serum, is the one which has amino-groups in its molecule. Those arsenical compounds which have the greatest therapeutic action are those which have amino-groups, and any interference with these groups at once diminishes the value of the drug. This is the reason why neo-salvarsan is not so potent as salvarsan, since one of the amino-groups has taken up a sodium formaldehyde sulphoxylate-group in place of a hydrogen atom. My experiments with the sulphur compounds show, not only that the therapeutic action of the drug is largely dependent upon its amino-groups, but also that that compound is best in which the amino-groups are in the ortho position. Although the amino-groups in salvarsan are in the ortho position, it must be remembered that they are only ortho to the hydroxyl-groups and not to the arsenic, to which they should be, if the maximum therapeutic action of the compound is going to be obtained. This is one of the reasons why intramine has a greater therapeutic action than salvarsan.

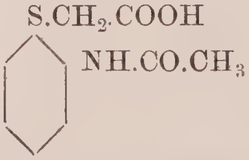
New Synthetic Anti-syphilitic Drugs.

Non-Metallic Compounds.

Sulphur.—I prepared two basic and two acid compounds. The first acid compound was the acetyl derivate of di-ortho-amino-thio-benzene and the second acid compound was the condensation product of the base with mono-chloracetic acid.

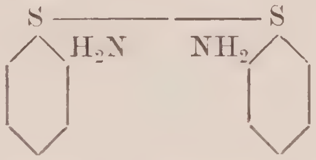


Acetyl derivative of intramine.

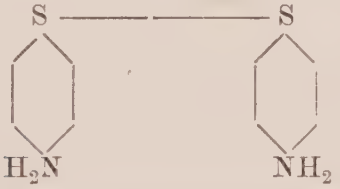


Condensation product of intramine with mono-chloracetic acid.

These two products may be quickly dismissed, as both were extremely painful when injected intramuscularly, and their therapeutic action was practically *nil*, which was to be expected since the amino-groups have been interfered with. The two basic compounds prepared were di-para-amino-thio-benzene and di-ortho-amino-thio-benzene. As the therapeutic action of the former was not as good as that of the latter it was discarded. The reason why the former is not as good as the latter is simply due to the fact, that the element is in a more powerfully adsorbed state, when the amino-groups are in the ortho position to it.



Di-ortho-amino-thio-benzene (Intramine).



Di-para-amino-thio-benzene.

Although I thought I was the first to prepare these sulphur compounds, I found, after my investigations had been concluded, that A. Hofmann[17] had prepared the di-ortho-amino body first in 1879, by oxidising ortho-amino-phenyl-mercaptan with ferric chloride, and that K. Hofmann[18] in 1894 had also prepared the same body by heating aniline with sulphur. Both father and son were working on the thio-anilines at the time, and neither prepared the substance for therapeutic purposes. This is practically the same as happened to Ehrlich, since the root compound of salvarsan was first prepared by Béchamp in 1863.

Intramine can be prepared as a mass which goes into a fine amorphous powder when dried, or as crystals; both are light yellow in colour with a greenish tint, and both are insoluble in water, but soluble in ether, alcohol, and acetone. In alcohol intramine readily crystallises out again, and in acetone the body soon breaks down, with the formation of aniline.

I have tried various media with which to make an emulsion of intramine, and liquid paraffin and olive oil appear to be the best.

Intramine can be very conveniently put up with lecithin. In this form the drug is loosely adsorbed, so that it forms a fine emulsion. Such an emulsion, owing to the small division of the particles, can be injected intravenously.* The intramine-lecithin compound as a 10 per cent. emulsion in water forms a perfect emulsion colloid, and the lecithin does not undergo auto-oxidation, owing to the strong antiseptic properties of the thio-body.

The next step was to test the compound biologically. So far as rabbits were concerned Ehrlich found that the *dosis tolerata* of salvarsan was 0·1 grm. per kilogram. Therefore, when I say that several times that amount of intramine can be injected into a rabbit weighing only 85 grm. it will be clear that intramine is an absolutely non-toxic substance. Of the numerous animal experiments made with intramine I have not lost one owing directly to the toxicity of the compound. Before it struck me to make a lecithin compound of intramine suitable for intravenous administration, I made some successful intravenous injections with an ethereal solution of intramine in rabbits, but, as the method used would cause some trouble in manipulation when applied to human beings, I discarded it. Repeated injections into the same rabbit caused no trouble, therefore I thought I was justified in trying intramine on human beings.

The first dose I injected was 0·45 grm. in 10 c.c. liquid paraffin, intramuscularly, to a man suffering from a well-marked papular syphilitic eruption. On the third day the rash was very much fainter, so I knew that I had a drug which had anti-syphilitic properties. I need not dilate further on the early results obtained, except to state that I found that as much as 12 grm. could be injected at one dose intramuscularly, and that 1–3 grm. doses could be injected daily intramuscularly with impunity.

When compared with salvarsan, I soon found out that in the primary and generalisation stages salvarsan was superior to intramine, but that in the recurrent stages the opposite was the case.

---

* Has only been tried on animals.

Further experiments showed me that, in the early stages of syphilis, previous administration of a metallic compound greatly increased the action of intramine, but that in the late stages the opposite was the case. When I came to realise that metals acted as oxidising agents and non-metals as reducing agents—a realisation fully justified when I found that salvarsan was better in early syphilis and that intramine was better in late syphilis, oxidation being more marked than reduction in the former, and *vice versâ* in the latter—I tried other metallic compounds, to see how their therapeutic action compared with that of salvarsan. The two metallic compounds used were the hydroxides of aluminium and iron. As both these hydroxides are suspension colloids, and therefore cannot be injected intravenously without producing anaphylactic shock unless calcium chloride is injected beforehand or the animal is anæsthetised with ether, etc., it was necessary to convert them into emulsion colloids, which I did with glycine, albumin, or gelatin. Injected intravenously into human beings as emulsoids, both the hydroxides of iron and aluminium caused an amelioration of the symptoms in cases of early syphilis, and enhanced the value of intramine injected afterwards, but they did not prove so valuable as salvarsan. This result was to be expected, since these hydroxides are not powerfully adsorbed bodies like salvarsan and intramine. Although when converted into emulsion colloids with glycine, albumin, or gelatin, amino-groups exist in the compound, there is no powerful adsorption between the metal and the amino-groups as there is in salvarsan and intramine.

Before describing the strongly adsorbed metallic compounds I prepared, it will be as well to dilate further upon the mode of action of intramine and to mention a few words about the history of sulphur as a reducing agent. Rey-Pailhade[23] discovered a ferment in 1888 to which he gave the name "philothion." This observer noticed that hydrogen sulphide was formed when finely divided sulphur was added to an alcoholic suspension of beer yeast. In 1889, Abelous and

Gérard[24] found a thermolabile ferment in animal organs which had the power of converting nitrates into nitrites, nitrobenzol into aniline, and of decolourising methylene blue. Abelous and Ribaut[25] thought that philothion was not a ferment, and therefore that their ferment had nothing to do with Rey-Pailhade's substance. Heffter[9] carried the matter further, and came to the conclusion that the reducing substance in extracts of animal organs was due to sulphydryl-groups, which only reduced certain bodies, and did not act as a ferment. Heffter found that substances which contained sulphydryl-groups, such as cystine, thio-glycocoll, thio-lactic acid, etc., decolourised methylene blue but did not reduce nitrates to nitrites or nitrobenzol to aniline. The last two reduction processes were considered by him to be caused by an aldehyde, an oxy-aldehyde, or by an amino-aldehyde. The next discovery in this direction was Schardinger's[26] enzyme, which threw no further light upon the subject.

Having already made several allusions to the *modus operandi* of intramine in this chapter, to save recapitulation I will begin with its action in the oxidase-reducase system. The oxidase-reducase system can be expressed in short as the hydroxyl-hydrogen system of the internal phase. On to the hydroxyl-group the metallic ion becomes attached; normally the metal is iron; when salvarsan is injected it is arsenic, etc. This metallic hydroxide combines with hydrogen ions and hydroxyl ions in the form of hydrogen peroxide, to form active oxygen, as *per* following equation:—

$$R.Fe(OH)_3 + 5H + 2H_2O_2 = R.Fe + 6H_2O + O''.$$

The iron-protein, which is formed as the result of the reaction, soon becomes re-converted into iron hydroxide-protein, as in the following equation:—

$$R.Fe + 4H_2O = RFe(OH)_3 + 4H + OH.$$

When intramine gets into the body, the compound presumably breaks down at the link between the two sulphur atoms. The sulphur then forms di-sulphide-protein, which reacts with the hydrogen and hydroxyl ions of the external phase, to form sulphur-protein, mercaptan-protein, hydroxyl ions in the form of hydrogen peroxide, and active hydrogen, as *per* this equation :—

$$R.S.S.R + 2H + 4OH = R.S + R.SH + 2H_2O_2 + H^{\cdot}.$$

In this way two atoms of peroxide hydrogen are formed, exactly the amount required for the metallic hydroxide to act upon, to form active oxygen.

Metals, then, form active oxygen directly, and non-metals indirectly.

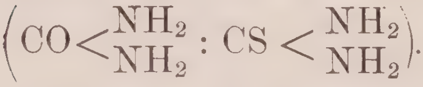
The mercaptan-protein is able to combine with any free sulphur, to form hydrogen sulphide and di-sulphide-protein, as in the following equation :—

$$2R.SH + S = H_2S + R.S.S.R.$$

The mercaptan-protein is also able to combine with any free oxygen, to form water and di-sulphide-protein, as in this equation :—

$$2R.SH + O = R.S.S.R + H_2O.$$

That the above is quite logical, follows from the relationship between oxygen and sulphur, as shown in such compounds as cyanates : thio-cyanates (R.CNO : R.CNS), and urea : thio-urea



$$\left(CO<\begin{matrix}NH_2\\NH_2\end{matrix} : CS<\begin{matrix}NH_2\\NH_2\end{matrix}\right).$$

Adding the knowledge just obtained to that gained by clinical observation, further light is thrown upon the affinity of mercaptan-protein for oxygen. If the mercaptan-group becomes oxidised by the active oxygen present, there will be less active oxygen resulting from the oxidising action of the metallic hydroxides, hence, if intramine is given first and salvarsan afterwards in early syphilis, when the greatest quantity of active oxygen is present, one would expect that those phases of the *Leucocytozoon syphilidis* which are most easily killed by oxidation would be able to flourish, that the customary therapeutic action of salvarsan would not be witnessed, and that the symptoms might even be temporarily aggravated.

This is exactly what happens clinically. The spirochætæ are not killed quickly by intramine, and, if salvarsan is given directly afterwards, it appears to have no immediate influence on the symptoms.

If the metallic compounds are allowed their full play first, as the catalysts of the oxidase system, the introduction subsequently of a strong reducing agent, like intramine, in virtue of its action, sets free sufficient peroxide to be subsequently converted into active oxygen by the metallic compounds, thereby preventing the adverse action of the mercaptan-protein.

This being so, it might be expected that the phases of the *Leucocytozoon syphilidis* would be quickly vanquished, and that the symptoms of the disease would rapidly disappear.

Once again this is exactly what happens clinically.

If metallic compounds be first administered in early syphilis, the spirochætæ are killed at once, and the symptoms improve. If intramine be now injected some of the other phases are killed immediately, and the symptoms vanish at a very much quicker rate than they otherwise would have done.

I need only cite one case to show the truth of this.

Case 3.—A patient had a typical Hunterian chancre in the corona, there was a moderate loss of surface and the sore was markedly indurated. I gave an intravenous injection of galyl (40 cgrm.), and three days later an intramuscular injection of intramine (3 grm.). There was practically no change in the chancre when the intramine was given, but a few days later, not only had the sore completely healed, but the induration had entirely vanished.

I have seen severe papular syphilides, scarcely influenced by galyl alone, disappear in a few days when intramine was injected, as the following case shows:—

Case 4.—The patient had a primary sore in the corona, hard infiltrative œdema of the whole of the penis, and a generalised papular syphilitic eruption. No improvement followed three intravenous injections of galyl, instead more papules appeared. Within ten days after one injection of intramine every lesion had vanished.

In the recurrent and late stages of syphilis, the exact opposite to what has just been said is to be expected, since in these stages the reducing action of the resisting substance is more powerful than the oxidising action.

One case again will suffice to prove this. *Case* 5.—A patient had a gumma which encircled the corona and spread on the under surface of the penis on to the scrotum. In less than one week after receiving only one intramuscular injection of intramine (2 grm.) the ulceration had completely healed. If this case had been treated with salvarsan alone, it would have taken about three weeks for the sores to have healed up, and during this time it would have been necessary to have given two or three intravenous injections.

I have treated with immediate success by intramine late syphilitic lesions which had remained practically uninfluenced by salvarsan, with mercury and iodides subsequently.

I must cite a case which bears this out to the letter.

Case 6.—A man 70 years of age had had syphilitic glossitis for 25 years. Eighteen months before he was given intramine, he had two intravenous injections of salvarsan, and these were followed by three courses of mercury and iodides, with practically no improvement in the clinical condition. When an intramuscular injection of intramine was prescribed (2 grm.), the tongue exhibited the following features. There was an ulcer in the centre about the size of a sixpence, with a lateral fissure running from it on both sides. Just posterior to the ulcer a hard wart was situated. The movements of the tongue were impeded and very painful. In a few weeks after the injection the ulcer had completely healed, the fissures and wart had vanished, the natural movements of the tongue and its suppleness had returned, and in the words of the patient "my tongue has never been so well as long as I can remember."

While on the subject of reducing agents, I will explain how iodine acts. Iodine has a more marked therapeutic action in late than in early cases of syphilis for the same reason that intramine has. Like intramine, iodine adsorbs hydrogen and hydroxyl ions to form peroxide for the oxidase system, as in the following equation:—

$$4I + 5H + 4OH = 4HI + 2H_2O_2 + H^{\cdot}.$$

The preparation of organic compounds of iodine, possessing a therapeutic action at all akin to that of intramine, is impossible, owing to the fact that iodine is a mono-valent element. Iodine increases the action of intramine, presumably because it is readily adsorbed by a mercaptan-group, thus checking the deleterious action of this group. The following formula shows the interaction :—

$$2R.SH + 2I = R.S.S.R + 2HI.$$

There is an interesting little point which is worthy of mention here, as the explanation of the phenomenon is forthcoming, and because it proves that metals and non-metals in these synthetic compounds act as catalysts.

I am referring to the spirillocidal action which the milk of a woman treated with salvarsan and salvarsanised serum exhibit.

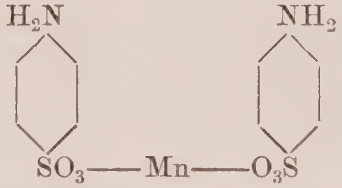
The activity of catalysts is phenomenal, for instance, the merest trace of platinum black, if added to a tank full of hydrogen peroxide, is sufficient to decompose the whole amount. In a small quantity of such a solution it is impossible to demonstrate the presence of platinum; therefore, if no arsenic can be demonstrated in the milk of a woman who has been treated with salvarsan, it is no proof that there is no arsenic present, as there may be ample for catalytic purposes.

Metallic Compounds.

Manganese.—In view of the experimental work which had been done in connection with the activation of oxygen by manganese hydroxide, I tried to see what effect strongly adsorbed colloidal compounds of manganese would have in syphilis. As the atomic weight of manganese is only 20 below that of arsenic (As = 74·96, Mn = 54·93), the therapeutic effect to be obtained from the use of the former would, on *à priori* grounds, be less than that from the use of the latter. On the other hand, it was possible that, if manganese was less toxic than arsenic, the loss of action might be compensated by using a greater quantity of the drug. Further work showed me that, if one metallic drug was less potent than another, the same therapeutic effect could be obtained by using a greater quantity of the former, but that there was no direct proportionality between the quantity used and the effect produced. This is an extremely important point, as it shows that the metals, and, for that, the non-metal sulphur, as in intramine, are catalysts.

As intramine is a drug which is best administered intramuscularly I thought it would be better, if possible, to prepare soluble compounds of the metals, so that they could be injected intravenously.

The first preparation made was the di-para-amino-manganese-benzene sulphonate.



Di-para-amino-manganese-benzene sulphonate.

Di-para-amino-manganese-benzene sulphonate forms a claret-coloured solution in water, it is acid and is readily broken down on the addition of an alkali.

When injected intravenously into rabbits, the animals die almost instantaneously from anaphylactic shock. The excess of hydroxyl ions in the blood doubtless breaks the compound down at once and manganese oxy-hydroxide ($MnO_2(OH)_2$) is formed.

$$Mn + 2OH + 2O = MnO_2(OH)_2.$$

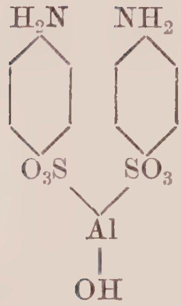
It will be noticed in the equation that two atoms of oxygen are used up, therefore, not only is the metal not acting as an oxidising agent, but it is increasing the hydrogen ion concentration, with the result that the animal dies at once. To avoid this, it is necessary to prepare the tri-para-body, since the moment the manganese becomes free, it can combine with three hydroxyl atoms to form manganese hydroxide ($Mn(OH)_3$), but unfortunately the tri-valent compounds of manganese are so very unstable. Before describing the other metallic sulphanilates, it would be interesting to point out the light which this work with manganese sulphanilate throws upon the penta-valent compounds of arsenic.

When arsenic becomes freed from a penta-valent compound, in the presence of hydroxyl ions it will become tri- and di-valent. The tri-valent ion will combine with three hydroxyl atoms to form arsenic hydroxide, in which form it can be adsorbed by protein and the metal rendered innocuous. The di-valent ion probably becomes converted into a tri-valent arsenic oxide (As_2O_3), which is toxic, hence the reason why such compounds as atoxyl and arsacetin are liable to produce optic neuritis. The toxic symptoms produced by the tri-valent adsorbed arsenic compounds are probably due to the formation of the para-amino-phenyl-arseno-oxide as suggested by Ehrlich, an oxide which is less toxic than arsenic oxide.

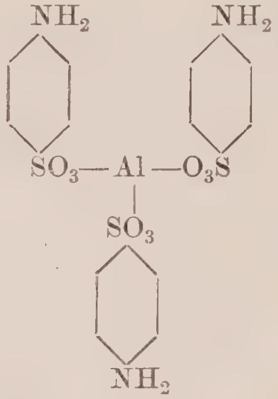
Aluminium.—Aluminium struck me as a useful metal to experiment with, as it can only form tri-valent compounds. Although its atomic weight is low, only 27, I nevertheless thought I might be able, if its compounds proved non-toxic, to achieve good results, by using very big doses. Aluminium is well known as an oxidising metal, Gessard[21] showed that the red oxidation product which results from the action of tyrosinase on tyrosine may be quickly converted into the black melanin by salts of aluminium. Bach[22] also showed that aluminium had the action of converting the oxidation product which results from the action of phenolase on pyrogallol into purpurogallin.

In passing, it may be interesting to note that melanin, the pigment in the epidermis, is formed by the action of tyrosinase on tyrosine. This action is an oxidase reaction and it is an action which is increased by metals. Syphilitic lesions, especially when they have been treated with arsenic, are very apt to leave pigment stains behind them, showing that the oxidase reaction is very marked in such lesions, and that it is increased by metals. This simple observation goes far to prove my theory that the syphilitic parasites are destroyed indirectly by oxidation, and that arsenic and some other metals act in virtue of their oxidising action.

The aluminium bodies prepared were the di-para-amino-hydroxy-aluminium-benzene sulphonate and the tri-para-amino-aluminium-benzene sulphonate, the formulæ of which are as follows:—



Di-para-amino-hydroxy-aluminium-benzene sulphonate.



Tri-para-amino-aluminium-benzene sulphonate ("Aluvine").

As the tri-para body, or, as it is commercially called, "aluvine," is the better of the two, I need not spend any time in describing the di-para or basic compound. Aluvine is soluble in water, in which it forms a colourless solution. Aluvine is acid and readily breaks down on the addition of an alkali; therefore when it is injected intravenously the metal quickly combines with three hydroxyl atoms, to form aluminium hydroxide.

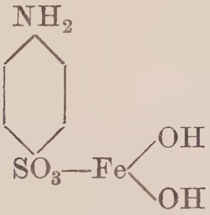
If a syringe is used for giving the injection, the blood which enters the syringe rapidly forms a flocculent precipitate, which is, in part, the metallic hydroxide. The observer might be alarmed at injecting such into the vein, but, as it is a perfect emulso-suspensoid, it is quite safe.

The method of injecting fluids intravenously is very much better than allowing the solution to enter by gravity, because if a suspensoid is present in the solution, the blood which enters the syringe will quickly give it an emulsoid coating. I am of the opinion that the reason why I have had so little trouble with the thousands of intravenous injections which I have given, is due to the fact that I use a syringe.

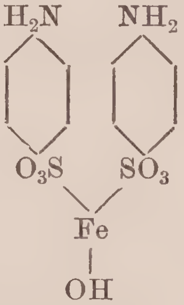
Aluvine is absolutely non-toxic, and, as far as I have been able to determine, any dose may be given, and it may be repeated as often as is desired. Patients can be treated as out-patients, because no immediate or late manifestations arise.

The first dose does not, as a rule, make much difference to the lesions, but within a few days after the second they quickly disappear.

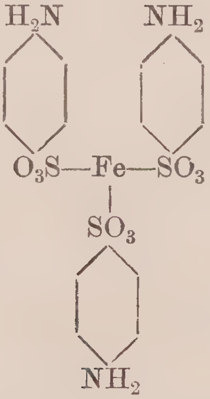
Iron.—As iron seemed to me to be the natural peroxidase, and having obtained such good results with intramine, a drug which increases the natural perhydridase, I anticipated good results from the use of an organic iron compound, and my anticipations have fortunately been realised. The iron bodies prepared were the para-amino-di-hydroxy-ferric-benzene sulphonate, the di-para-amino-hydroxy-ferric-benzene sulphonate, and the tri-para-amino-ferric-benzene sulphonate, the formulæ of which are as follows:—



Para-amino-di-hydroxy-ferric-benzene sulphonate.



Di-para-amino-hydroxy-ferric-benzene sulphonate.



Tri-para-amino-ferric-benzene sulphonate ("Ferrivine").

Similar to the aluminium body, the tri-para compound of iron appears to be the most suitable. It is a crystalline body, soluble in water, to which it imparts a deep orange-red colour. Ferrivine, as the tri-para compound is commercially called, is non-toxic, and it has a very marked action upon syphilitic lesions. Ferrivine is supplied as a 1-per-cent. solution, *i.e.*, 100 c.c. or 3½ ozs. contain 1 grm. of the salt. The solution is stable, it does not oxidise in the air, and it can be diluted with distilled water if desired. The dose suggested may be repeated at frequent intervals without risk. The dose I usually employ is 1 grm., but I sometimes inject 2 or 3 grm. The only disadvantage which I have so far experienced with ferrivine is that the patient sometimes feels faint immediately after the injection. Recovery sets in in a few minutes, when the patient can do as he likes. The collapse occurs because the normal hydrogen ion concentration is upset.

The following case will give the reader a good idea of the symptoms which are met with in an extreme case :—

Case 7.—The patient complains of pain at the site of injection, the vein for about an inch of its course appears swollen and becomes distinctly blue in colour. The patient then gets red in the face, and the eyes look as if they were bloodshot. A buzzing sound in the ears and a whirling sensation in the head are the next symptoms to ensue. The patient then experiences difficulty in breathing and finds he cannot talk ; at this stage he thinks he is going to choke and die. Pain over the heart and especially in the pit of the stomach sets in. As the other symptoms are disappearing and the patient's voice returns, pallor follows the redness, and the pain complained of at the lower end of the sternum increases in intensity. The symptoms then pass off one by one, and about half an hour later the patient feels all right.

The pulse rate at first becomes very rapid, then feeble, until it can scarcely be felt. As the symptoms disappear the pulse gradually becomes normal.

These changes are, of course, due to a temporary but marked upset of the normal hydrogen ion concentration of the serum. The upset occurs because the iron, not being very strongly adsorbed, soon becomes free when the acid compound comes in contact with the alkaline serum. Iron, being tri-valent, quickly adsorbs three hydroxyl atoms, with the result that the hydrogen ion concentration is temporarily raised. Collapse only occurs when very big doses are used, and it is not so dangerous as that which follows arsenic, because the molecular weight of iron is less. Experiments are in hand to overcome this disadvantage, and they already forebode success.

Methods of Treatment.

General Considerations.—It would be as well now to give a sketch of the clinical value of these new drugs, and how they should be used with the already known remedies in the treatment of syphilis.

Aluvine and ferrivine act like salvarsan, but in some cases with more effect; being non-toxic, larger doses can be given, and they can be repeated at shorter intervals. Generally speaking, the symptoms of primary and generalised syphilis disappear within four days after the second intravenous injection. Induration in chancres, and those dense infiltrated brownish papules, are not quite as resistant to aluvine and ferrivine as they are to salvarsan, and, if intramine be prescribed after the second injection, the hardest induration and the most resistant papule will vanish within a fortnight. I had a case of generalised syphilis in which the papules not only did not move under galyl and salvarsan, but more appeared, while the arsenical preparations, mercury, and iodides were being administered. Five days after an injection of intramine, every papule had vanished. One of the best results so far obtained with ferrivine was the case of a man, who had three chancres on the penis. On a Tuesday 3 grm. of ferrivine were injected intravenously. On the following Saturday the chancres had completely healed. In the recurrent and late stages of syphilis, intramine should always be prescribed first, when the symptoms vanish in a few days. In cases of cerebro-spinal meningitis the pains disappear within 24 hours, in degenerative myelitis the subjective symptoms are often improved, and I have also seen considerable benefit follow intramine in cases of degenerative encephalitis. Interstitial keratitis clears up with intramine in a most marvellous way.

I will describe a case of degenerative encephalitis treated with intramine, as it shows so well what can be done by combining reducing and oxidising remedies.

Case 8.—The patient was suffering from the depressive type of degenerative encephalitis, he showed no change of facial expression and would not speak, he had no control over his sphincters, and was at times violent. I prescribed two intramuscular injections of intramine, with the most extraordinary improvement—facial expression returned within a few days, and the patient entered into conversation. Hoping that the condition might be still further improved, a third injection was given, with the result that the patient became worse than he had ever been. I had suggested that, after the second injection of intramine, a metallic compound should be administered, but, as the medical man in attendance wished to see what intramine alone would do, it was not prescribed. Seeing that the patient had become worse after the third injection, which was what I expected, I hazarded the opinion that, if a metallic compound was given now, his improvement would be sudden, and greater than on the first occasion. An intravenous injection of galyl was administered, with the result that in 48 hours' time the patient became a rational individual. For how long the improvement is going to be maintained I cannot say, but this case is especially interesting to me as a proof of my theory of oxidation and reduction. Three months have now elapsed since the patient had his last injection, during which time a steady improvement has been maintained. The patient has returned to his business. Before he came under treatment he could not write. With salvarsan I have seldom seen a case of degenerative encephalitis improve, and I have seen many made worse.

As the maximum therapeutic action is obtained in early syphilis if intramine follows the administration of aluvine or ferrivine, likewise is it the case, if in late syphilis one or other of the metallic compounds follows intramine. I have treated some cases of cerebro-spinal meningitis with, first an intramuscular injection of intramine, then with two intravenous injections of a metallic compound, and finally with mercury and iodides, with the very greatest success, so much so that I think such patients are better advised to have the treatment just outlined in preference to intraspinal injections of salvarsanised serum.

Owing to the non-toxicity of intramine, aluvine, and ferrivine, serum withdrawn after their administration and injected intraspinally has a most beneficial influence upon central nervous lesions, superior to that of salvarsanised serum, and, moreover, no toxic symptoms occur, such as are too often witnessed when galyl and the other arsenical substitutes for salvarsan are used.

The following case is one in point :—

Case 9.—A man, aged 34, came to me complaining of double vision. He had contracted syphilis five years previously, and had been treated thoroughly with mercurial inunctions and injections before the symptoms of the generalisation stage manifested themselves. The patient had also had nine intravenous injections of salvarsan at various periods, but had never had another symptom of syphilis. On examination, I found that there was a unilateral partial third nerve paralysis, the pupils were unequal, and the reflexes were sluggish. The patient had no knee-jerks, and there were areas of diminished tactile sensation. I elicited from the patient that he had lost much weight, and that all sexual desire had vanished, in the last few months.

I gave three intramuscular injections of intramine, and alternated them with three intravenous injections of aluvine. After each injection I gave an intraspinal injection of the prepared serum. The cerebro-spinal fluid, when first examined, exhibited the typical features of degenerative myelitis, after the fifth injection it was normal, and by this time all the objective and subjective symptoms had vanished. The double vision disappeared after the first injection of intramine, showing clearly that the cranial nerve lesions in degenerative myelitis must be of meningeal origin, commencing in the meninges covering the nuclei in the base of the brain.

The introduction of a reducing agent like intramine into our therapeutic armamentarium is going to have a very marked effect in combatting not only syphilitic lesions, but also lesions of possibly all chronic infections. The following case will show its influence in the former : *Case* 10.—A medical man, aged 42, contracted a digital chancre nine months ago. In all he had seven intravenous injections of neo-salvarsan and galyl, the last two injections of galyl being given after the appearance of a big recurrent syphilitic ulceration of the right tonsil, but without any therapeutic action. The patient had taken some mercury, but rather irregularly. I gave an intramuscular injection of intramine, in a few hours the subjective symptoms in the throat vanished, and by the fourth day it would have been difficult to say that the tonsil had been ulcerated. The improvement in the general health was just as remarkable.

Cases of lupus, which Dr. Adamson has treated with intramine, upon which a report will appear shortly, show its influence in the latter. The therapeutic effect produced has far surpassed my most sanguine expectations. In a few days ulcers heal and the granulomatous masses become flush with the surrounding skin, a change which often cannot be wrought in months and even years by Finsen light. I have also had some success with intramine in the treatment of chronic cases of gonorrhœa,* and also in the treatment of chronic ulcers of non-syphilitic origin. Old standing varicose ulcers, which have resisted treatment for years, begin to mend in a few days. Intramine possesses very strong antiseptic properties, therefore it is extremely valuable as a dressing for wounds.

Although I have not yet used ferrivine and aluvine on quite such a large scale as intramine, I have given sufficient injections to state the fact, that the need for the use of a toxic metal like arsenic will, as the iron compound is improved, no longer remain.

The future only will show what effect these new drugs are going to have upon the cure of syphilis and upon the incidence of recurrences; therefore there is no point in discussing this problem now.

In outlining the treatment I now adopt in the various stages of syphilis two very important questions arise, the correct answers to which it is necessary to find.

---

* Intramine injected into the urethra causes a great increase of soluble phosphates and carbonates in the urine. These salts are more marked in the first glass than in the second glass of urine passed, showing that they are produced locally. Phosphates and carbonates are the salts which regulate the normal hydrogen ion concentration of the external phase; therefore this observation proves beyond doubt that intramine acts on the oxidase-reducase system.

(1) Assuming that in the doses given the oxidising effect of salvarsan, aluvine, and ferrivine respectively, is equal, is that effect going to be enhanced by giving an injection of each, or is the effect going to be the same if three injections of any one are prescribed ? Theoretically I should doubt whether any difference in effect would be produced, but whether this is the case practically remains to be seen—a point by no means easy to determine.

(2) If we fail in curing a case of syphilis at once, are we right in continuing treatment after the disappearance of the symptoms, or might it not be better for the patient to be treated symptomatically only ?

The syphilitic parasites are attacked first and foremost by the resisting substance, *i.e.*, the lipoid-globulin molecules in the serum. The resisting substance has an oxidising and reducing action, actions which we increase by salvarsan, aluvine, and ferrivine on the one hand, and by intramine on the other.

An increase in size of the lipoid-globulin molecules, produced by the administration of the synthetic remedies just mentioned, results in a breaking down of the molecules, with ultimately a diminution in the amount of the resisting substance.

The properties of the resisting substance may be lessened, not only to below those of the resisting substance in bacterial diseases, but also to below those of the normal resisting substance. Therefore, the continued administration of powerful oxidising and reducing remedies would result in so little of the compound being adsorbed, that their oxidising and reducing actions would be reduced to perhaps *nil*. Knowing that both oxidation and reduction are catalytic reactions, it might theoretically be possible that only the minutest traces of the metallic hydroxide and the di-sulphide are necessary to continue the good work. Since mercury presumably acts mechanically, in virtue of its high atomic weight, and less as a peroxidase than metals with a lower atomic weight, and considering that its action is entirely dependent upon the size and adsorptive capacity of the lipoid-globulin molecules, it might theoretically be assumed that the administration of mercury, once the properties of the resisting substance had been reduced to, or below par, would be entirely superfluous.

Ever since the advent of salvarsan I have been struck by the enormous increase of cases of meningeal syphilis and the very frequent occurrence of lesions, in spite of the fact that the patient was well under the influence of mercury. I have also seen many cases in the latent stage precipitated into a condition of degenerative myelitis and degenerative encephalitis by the administration of our powerful remedies. So far as dealing with patients in the latent stage is concerned, I have for some time made up my mind never to prescribe treatment, if I found no symptom after a thorough clinical examination. I have also long since made up my mind to treat only symptomatically those patients who have once entered the recurrent stage, but as to how a case in the generalisation stage should be best treated, a little further discussion is necessary.

To make the subject clear I will picture the body as consisting of two parts, (1) the systemic part, (2) the nervous. The nervous part is largely dependent upon the systemic part for its resisting substance.

If the resisting substance in the systemic part has been reduced to, or below par by treatment, spores which are resistant to treatment may be possibly prevented from developing, if small doses of the remedies are continually being prescribed. The rule is far from being invariable. Against this one must consider the frequency of recurrences when mercury and iodides are not prescribed after a course of several injections of salvarsan, and how the protective action of mercury and iodides compares with that of the resisting substance when working alone.

Systemic recurrences are far more common when salvarsan is not followed by mercury and iodides, but the frequency of the same and when they appear, depend upon the number of injections given. If three or less injections of salvarsan are given, the recurrences are more frequent than when six or more injections are given in one course of treatment, but they appear much later, usually 12 or 18 months have elapsed before they make their appearance, while after a course of several injections a recurrence may appear in two or three months. Unless the individual has the opportunity of observing many cases, this somewhat paradoxical result may easily lead to false conclusions being made.

The explanation of this disparity as to the time at which the recurrences appear is as follows: When three injections of salvarsan are prescribed, the resisting substance is scarcely reduced, with the result that it continues exerting its power until the parasites ultimately get the upper hand, which is hardly ever under 12 months. When several injections have been administered, the resisting substance is so reduced that the spores which have not been killed by the treatment will be able to develop on a soil which to them is the same as a new soil, consequently symptoms will appear after the usual incubation period, therefore two or three months later. The early syphilitic lesions are far less dangerous and cause much less local damage than late syphilitic lesions, hence the systemic recurrences after a course of several injections are preferable to those which occur when only three injections have been given, since the former produce fewer degenerative changes, especially in the vessels, which in all lesions are primarily attacked.

Therefore, a course of several injections produces better results than a course of a few injections, and since the recurrences after both are fewer in number when the injections are followed by mercury and iodides, these two drugs should not be omitted in the treatment of syphilis.

As mercury and iodides can have such an infinitesimal action when the resisting substance is at, or below par, might it not be better for the patient after a course of several injections to wait six to twelve weeks before taking mercury? Six to twelve weeks is about the time the resisting substance takes to get back to its normal state. Now mercury and iodides have nothing like the influence in altering the state of the resisting substance that the strongly adsorbed compounds have, and, since both may have some power in preventing the spore from developing, it would seem wiser to allow no interval to elapse between stopping the synthetic metallic compounds and commencing the mercury. Whether mercury should be given continuously or intermittently does not, I think, matter much. As mercury in the form of injections is more powerful than when taken *per os,* from the patient's point of view the intermittent treatment is the only one which is practicable.

A question, which is impossible to answer is, how long the intermittent treatment should be maintained. A correct answer could only be given, if we knew that a spore would not develop if it had been dormant for a certain number of years. A spore may remain dormant for 50 years, then to re-develop and to cause symptoms, therefore a definite answer will never be forthcoming. Furthermore, we do not know what influence treatment has upon dormant spores, nor do we know whether treatment postpones their recrudescence. In this uncertainty it is best to let our clinical experience guide us, and to give one year's treatment when the case of syphilis is in the primary stage, and two years' treatment in the generalisation stage.

Owing to the fact that the nervous part is dependent for its protective substance upon the systemic part, it will be readily understood that if the organisms have reached the nervous part before treatment has commenced, they will be able to develop therein the more readily, the quicker the resisting substance in the systemic part has been reduced, provided at the same time the organisms in the nervous part are not vanquished by the treatment. It will follow, therefore, that the incidence of cerebro-spinal meningitis will be greater when many injections of salvarsan, than when a few have been prescribed, since it is difficult in every case to give injections sufficient to kill the organisms in the nervous part. Cerebro-spinal meningitis is a curable condition, and, moreover, its occurrence practically precludes the patient from getting a degenerative lesion later.

Now let us consider what happens to the nervous part if only a few injections of salvarsan are administered. The resisting substance is not strong enough to kill the parasites, but strong enough to prevent them developing sufficiently to produce symptoms. In this state, the spores can and do migrate into nerve tissue proper, where they meet with still less resistance, with the result that now any symptoms which appear will be degenerative in character, and therefore more or less incurable. Once again we see that a course of several injections is preferable to a course of only a few.

The onset of nervous lesions is naturally dependent upon the number of organisms that reach the meninges before treatment is begun. Cerebro-spinal meningitis is much more common in patients who have severe generalised symptoms than in those in whom the so-called "secondaries" are slight. For all practical purposes, it may be said that a ratio exists between the severity of the early cutaneous eruptions and the occurrence of early cerebro-spinal meningitis.

As the symptoms of cerebro-spinal meningitis almost invariably begin to manifest themselves between 8 and 12 weeks after the last injection of salvarsan, it is wise to give every patient in the generalisation stage an extra course of powerful treatment round about this time.

What effect the introduction of intramine is going to have upon the points just raised, I am not in a position to say, but one thing is clear, namely, that the administration of intramine reduces the number of the long course of the metallic compounds by about half, and increases the result produced.

As iodides increase the action of intramine, possibly owing to the fact that the mercaptan-protein will react with iodine as it does with oxygen, thereby saving the free oxygen which is so necessary, with the result that the iodine converts it back into di-sulphide protein, I often prescribe iodides one week before and one week after the administration of any big dose of intramine.

The equation of the interaction is probably as follows:—

$$2\mathrm{R.SH} + 2\mathrm{I} = \mathrm{R.S.S.R} + 2\mathrm{HI}.$$

Primary Stage.—In the primary stage I prescribe iodides first of all, and inject intravenously a large dose of a metallic compound (1–3 grm.), which I repeat one or two days later. Three days after the second injection I give an intramuscular injection of intramine (1–2 grm.), and, between the fourth and seventh day after, I inject intravenously another maximum dose of a metallic compound, and continue mercury, intramine and iodides for one year, as follows :—

1. Eight fortnightly intramuscular injections of grey oil, 2–3 gr. of Hg.
2. Eight fortnightly intramuscular injections of intramine, 1–3 c.c.

Mercury and intramine alternately.

3. One month in every three, iodides should be prescribed. The first month of iodides should be commenced after four doses each of mercury and intramine have been injected.
4. One month's rest should follow every eight injections each of mercury and intramine. Injecting mercury and intramine alternately, the former is prevented from producing salivation, tender gums, and local accumulations in the buttocks.

As an alternative method for carrying out the mixed treatment I suggest the following :—

1. Eight weekly intramuscular injections of grey oil, 1 gr. of Hg.
2. Eight weekly intramuscular injections of intramine, 1 c.c. Mercury and intramine alternately half-weekly instead of weekly.
3. After the injections, iodides for three weeks.
4. Rest from treatment for five weeks.

Concerning that part of the treatment dealing with iodine I should like to make a few remarks.

The disadvantages attaching to the internal administration of iodides are familiar to everyone, and they cannot be said to have been surmounted by the various organic preparations which have come lately into vogue.

I have recently carried out a series of experiments with Crookes' colloidal iodine (1 in 500), and have found that it can be injected intravenously, intramuscularly, and taken internally with impunity. Intravenously, 100–300 c.c. are well borne. Intramuscularly, only small doses can be given because the bulk provides a limitation. Internally, one to four teaspoonfuls can be taken two or three times a day about two hours after meals.

If I am prescribing iodine before and after an injection of intramine I sometimes inject intravenously 100 c.c. of collosol iodine on the same day or the day before the intramuscular injection, and repeat it four or five days later.

Generalisation Stage.—In the generalisation stage, I prescribe iodine at once, and two or three intravenous injections of a metallic compound both before and after an intramuscular injection of intramine, allowing two days to intervene between each intravenous injection. I then give four or six courses of the mixed mercury, intramine, and iodine treatment, and two months after the last intravenous injection I inject intravenously a maximum dose of a metallic compound before and after a large intramuscular injection of intramine.

In all recurrent and late cases, I first of all give one or two intramuscular injections of intramine, then one or two intravenous injections of a metallic compound, and follow up the same with one course of the mixed treatment. This treatment will also do for cases of degenerative and non-degenerative meningo-encephalitis and -myelitis. In certain cases of degenerative myelitis in which more drastic treatment might be expected to give a more permanent result, I give the following:—

1. An intramuscular injection of intramine.
2. Four days later, an intraspinal injection of prepared serum which has been drawn off on the third day.
3. Three or four days later, an intravenous injection of a metallic compound. The metallic compound should not be galyl, kharsivan, neo-kharsivan, or any of the other French substitution products for salvarsan, as they are too toxic.
4. Three days later, an intraspinal injection of prepared serum which has been collected the day before.

The injections of the non-metallic and metallic compounds should be continued alternately until the cerebro-spinal fluid is normal or approaching close thereto. I have had so much success in both degenerative encephalitis and myelitis, by alternating intramine with a metallic compound, that I am wondering whether intraspinal injections will ever be necessary.

Syphilis in Women.—Women who are pregnant should be treated as first described under the heading of the generalisation stage, and two courses of the mixed treatment should be given, without any pause in between. The extra injections at the second month are unnecessary.

Congenital Syphilis.—Congenital syphilitics should have the mixed treatment for two years, with this difference; that the mercury should be adminstered *per os*, and continuously. All recurrent symptoms should be treated symptomatically, and intravenous injections prescribed if practicable, as suggested in recurrent and late cases of acquired syphilis.

As intramine is a new drug, some points with regard to its administration might with advantage be given. All intramuscular injections are apt to be painful; the intensity of the pain varies with different individuals, and is mostly due to the bulk of the injection and the œdema and contraction of the muscle fibres which follow it.

There are several ways in which the pain can be reduced. First and foremost the intramine should be well warmed before it is injected. Immediately after the injection pain is experienced, this lasts for a few minutes, then vanishes, to reappear some hours later when the œdema and contraction set in.

The part should be well massaged after the injection has been given, the patient should walk about to aid absorption, and, the moment the stiffness begins to set in, he should have a very hot bath in which some soda has been dissolved. For the next two or three days, the leg should be moved as much as possible, hot baths should be repeated, and the part should be well massaged. By the fourth day every trace of intramine will have vanished from the buttock, no induration will remain, and the patient should be able to move about in the normal way. There is usually a rise of temperature on the second and third days, the degree depending upon the severity of the case; the patient may perspire freely, and sometimes, a few days later, an erythema appears over the injected buttock, which either remains localised or spreads over the body, in every case to disappear 24 or 48 hours later. The erythema is probably due to the sulphur.

However big be the dose and however frequently it be repeated, I have never noticed any other ill-effects than those already described; therefore it may safely be assumed that intramine is an entirely innocuous drug.

I cannot close this subject without the prophecy that intramine, especially when alternated with powerfully adsorbed oxidising agents, of which ferrivine is the best, is going to play a very important part in the treatment not only of syphilis, but of all chronic infections, since it has already proved itself to be of considerable value in cases of tuberculosis, chronic gonorrhœa, and chronic ulceration. It is even probable that intramine may have a place in the treatment of malignant disease, and practically certain that in leprosy it will prove invaluable.

I have little doubt but that malignant disease is also attacked by oxidation and reduction. The selective action of radium, etc., on malignant cells will, I expect, ultimately be proved to depend upon a simple process like oxidation and reduction, or upon the influence radium has upon surface energy, in virtue of its high atomic weight. If this proves to be the case, the action of radium in malignant disease will be analogous to the action of mercury in syphilis. By the way, is the action of radium a selective one on malignant cells, or does it act in virtue of its effect upon the lipoid-globulin molecules, or the protective substance of the serum, like chemotherapeutic agents in general seem to do?

Having had other proofs of my theory as to the mode of action of chemotherapy I will proceed to state what they are. Chilblains are, in all probability, due to insufficient oxygenation of the tissues of the extremities. If true it is to be expected that if the amount of active oxygen could be increased locally, they would immediately disappear. That such is the case the following will show:—

Case 11.—A man, aged 35, had extremely bad chilblains on the hands, feet, and ears. Prior to the winter of 1915–16 he had never suffered from them. The chilblains began in

November, 1915, and persisted till March, 1916, in spite of the different remedies which had been used. They made no sign of fading until collosol argentum (1 in 2000) was rubbed into them. The application caused an immediate and temporary aggravation, but within 24 hours all the chilblains had disappeared.

I have had other equally successful cases, in which one application of a colloidal metallic preparation has been sufficient to cause their disappearance.

It is highly probable that piles are in part due to local want of active oxygen, since suppositories of the metallic collosols undoubtedly prove beneficial. The particles of colloidal silver still retain their Brownian movements in the depth of the gelatin, which is used as the base for the suppositories.

If sections of wounds, ulcers, etc., which have been stained with pyronin and methyl green, are examined microscopically, they will be seen to fall into two groups: (1) those the cells of which show a special affinity for methyl green; (2) those the cells of which show a special affinity for pyronin. The cells of the former are characterised by their richness in nuclei. Nuclei are rich in active oxygen, hence the affinity for methyl green. The sections which show an affinity for methyl green come from new or acute cases, therefore new wounds or acute ulcers are especially rich in active oxygen. This being the case, it is to be expected that remedies which increase the amount of active oxygen will quickly cause reparation of wounds, the protective cells of which are already doing their best with the amount of active oxygen at their disposal.

Clinically this expectation is realised, because if colloidal silver is applied as a solution or in a gelatin paste to acute wounds or ulcers they quickly heal. If the repair is not as rapid as anticipated it may be quickly hastened by the application of a non-metallic colloidal preparation, such as intramine. If sections which are pyroninophile are further examined it will be noticed that the cells are richer in protoplasm than in nuclei, that their reducing action is greater than their oxidising action, and that such sections come from chronic wounds and ulcers.

In these cases the application of a reducing agent would on *a priori* grounds be expected to have a more beneficial action than an oxidising agent. The application of collosol argentum to a chronic soft sore, for instance, is of no value at all, while one application of intramine will within 48 hours heal an ulcer which has persisted for weeks. The local application of intramine is painless, it stimulates epithelial growth more rapidly than scarlet red, and, in addition, it causes a large local collection of lymph. Intramine is the best lymph producer I have ever seen, therefore it should prove invaluable in some of the shrapnel and bullet wounds. The following case may prove of interest:—

Case 12.—A man, aged 42, consulted me for some ulcers he had on his leg. On one leg were three ulcers, each about the size of a shilling, which the patient had had for about six months. The ulcers were typical gummata, although the patient was unaware that he had ever had syphilis. On a Friday evening I injected 1·5 grm. of intramine into the buttock and also dressed the ulcers with the drug. The intramine dressings alternated with colloidal silver were changed twice daily and kept on for 12 hours at a time. By the following Thursday the ulcers had completely healed. The intramine injection was followed by two of ferrivine.

The theory that diseases are combatted by drugs which simply increase the amount of oxygen directly or indirectly is, I think, fully proved, with the result that treatment is now put upon a logical basis and empiricism is removed. The paths opened up for future research are infinite, the treading of which must always lead to some substantial progress being made and not to a further penetration into darkness, the direction taken by so much of the research work done in medicine in recent years.

The theory of oxidation and reduction has not only explained directly the *modus operandi* of chemotherapy, but also indirectly that of immunity, with the result that the *rationale* of dyeing and staining, of the immunity reactions, and of anaphylaxis is rendered clear. We should now be able to discard the hundreds of coined words which have never served a useful purpose, and to replace irrational tests by rational ones. So far as syphilis is concerned a rational test has been discovered, which is to be hoped will take the place of the Wassermann reaction—I am referring to the E-S. test, which has been fully described in Chapter VII.

REFERENCES.

[1] Ehrlich u. Bertheim (1907), “Berichte der deutsch. chem. Gesell.,” xl, 3292.
[2] *Ibid.* (1908), xli, 931, 1672.
[3] *Ibid.* (1910), xliii, 924.
[4] Ehrlich u. Hata (1910), “Die experimentelle Chemotherapie der Spirillosen.” Springer, Berlin.
[5] Ehrlich u. Gonder (1913), “Handbuch der pathogenen Mikroorganismen,” iii, 337.
[6] Ehrlich (1912), “Zeitschr. f. Chemother.,” i, 1.
[7] McDonagh (1915), “The Biology and Treatment of Venereal Diseases” (Chapter vi). Harrison and Sons, London.
[8] Pighini (1912), “Bioch. Zeitschr.,’ xlii, 124.
[9] Heffter (1908), “Arch. f. exper. Pathol.,” Festschr. f. Schmiedeberg, Suppl. Band, 253.
[10] McDonagh (1915), *ibid.* (Chapter x).
[11] Béchamp (1863), “Compt. Rend. de l’Acad. des Sci.”
[12] McDonagh (1915), “Practitioner,” xcv, 757.
[13] Hardy (1914), “Surface Energy.” London (in preparation).
[14] Ishizaka (1913), “Zeitschr. f. physik. Chemie,” lxxxiii, 97.
[15] Freundlich u. Schucht (1913), “Zeitschr. f. physik. Chemie,” lxxxv, 641.
[16] Hügel (1913), “Archiv. f. Derm. u. Syph.,” cxviii, 1.
[17] A. Hofmann (1879), “Berichte der deutsch. chem. Gesell.,” xii, 2363.
[18] K. Hofmann (1894), “Berichte der deutsch. chem. Gesell.,” xxvii, 2807.
[19] Secretary, Nat. Med. Res. Com. (1915), “Brit. Med. Journ.,” i, 897; “Lancet,” i, 925.
[20] *Ibid.* (1915), “Brit. Med. Journ.,” i, 1087; “Lancet,” ii, 1198.
[21] Gessard (1900), “Compt. Rend. de l’Acad. des Sci.,” , 1327.
[22] Bach (1910), “Berichte der deutsch. chem. Gesell.,” xliii, 366.
[23] Rey-Pailhade (1888), “Compt. Rend. de l’Acad. des Sci.,” cvi, 1683.
[24] Abelous et Gérard (1889), “Compt. Rend. de l’Acad. des Sci.,” cxxix, 164.
[25] Abelous et Ribaut (1903), “Compt. Rend. de l’Acad. des Sci.,” cxxxvii, 95.
[26] Schardinger (1908), “Chem. Zeitung,” xxviii, 704.

CHAPTER X.

THE SIGNIFICANCE OF THE HYDROGEN AND HYDROXYL IONS IN BIOLOGY.

ALTHOUGH much of what I have to say has already been mentioned in other chapters, the subject is so important, forming as it does the basis of immunity and chemotherapy, that I think a useful purpose will be served by devoting a special chapter to the *rôle* played by the hydrogen and hydroxyl ions in biology.

One of the oldest known facts in electricity is the decomposition of water by an electric current into hydrogen and oxygen.

Another equally well known fact is that in every aqueous solution, be it acid, alkaline or neutral, there must be hydrogen and as well hydroxyl ions—the acidity or alkalinity of the solution depending upon the prevalence of either one of these ions.

It is not quite so well known that water is an exceptional substance, a fact clearly seen when it is stated that the hydrogen and hydroxyl ions, of all the ions known, have by far the greatest rate of dispersion.

For instance at ordinary temperature the rate of dispersion of the hydrogen ion is almost five times, and that of the hydroxyl ion three times, as great as that of the quickest rate of dispersion of any other ion known.

Rate of Dispersion of Various Ions. Temperature 18°.

$H^{\cdot}$.	OH'.	$K^{\cdot}$.	$Na^{\cdot}$.	Cl'.	CH_3COO'.
318·0	174·0	64·6	43·5	65·5	40·8 (25°).

(After Kohlrausch.)

The hydrogen and hydroxyl ions are further exceptional in that they possess an extraordinarily high chemical affinity for one another, so that whenever free ions exist together, both join to form water, but not all the ions are bound, as there are always some left free.

An acid is a substance which in an aqueous solution forms hydrogen ions. A substance which forms both hydrogen and hydroxyl ions is spoken of as an amphoteric electrolyte or, simply, as an ampholyte.

The acid substance dissociates in water to form a negative electrolyte and a positively charged hydrogen ion

$$HCl \rightleftarrows H^{\cdot} + Cl'.$$

The basic substance dissociates in water to form a positive electrolyte and a negatively charged hydroxyl ion

$$NaOH \rightleftarrows Na^{\cdot} + OH'.$$

From this it follows that the positive charge will increase with an increase in acidity of the liquid, and *vice versâ*. Therefore in alkaline solutions there are more hydroxyl and less hydrogen ions than in water. Since serum is on the alkaline side of neutrality it will follow that there is always an excess of hydroxyl ions (OH').

Serum is peculiar in that the body maintains in it a normal equilibrium between the hydrogen and hydroxyl ions—an equilibrium which is commonly known as the normal hydrogen ion concentration. The hydrogen ion concentration of serum is $0{\cdot}4 \times 10^{-7}$ and the corresponding hydroxyl ion concentration is $7{\cdot}2 \times 10^{-7}$—an excess of the latter, as is seen, owing to the fact, as already stated, that serum is always on the alkaline side of neutrality.

Two questions now arise: (1) For what purpose is this normal hydrogen ion concentration? (2) How is this normal hydrogen ion concentration maintained?

One of the most important *rôles* played by the hydrogen ion concentration is the influence it exerts upon the solubility of insoluble electrolytes. As is well known, free acids are more insoluble than their alkaline salts, and free bases are more insoluble than their chlorhydrates. Take, for instance, the base of salvarsan, namely, di-ortho-amino-arseno-benzene, or the base of intramine, namely, di-ortho-amino-thio-benzene: both are insoluble, or, in other words, cannot undergo electrolytic dissociation, yet, when injected, electrolytic dissociation must occur, as otherwise they would exert no therapeutic action. In the case of the di-ortho-amino-arseno-benzene, by inserting into its molecule two hydroxyl-groups, electrical dissociation will occur in water, where there are free hydrogen and hydroxyl ions, and the dissociation will be greater if a salt of the base is used. Hence the reason for the insertion of the hydroxyl-groups in salvarsan and the formation of the hydrochloride, two steps which really are not necessary, so far as the therapeutic action of the drug is concerned.

Before an explanation as to how electrical dissociation of an insoluble body like intramine occurs *in corpore* it will be as well to show how electrical dissociation of a simpler body occurs. The solubility of benzoic acid in water, for instance, can be followed by the following equations taken from an article by Michaelis[1] in the *Handbuch der Biochemie*:—

$$\frac{[\text{Acid anions}] \times [H^{\cdot}]}{[\text{Non-dissociated acid}]} = k.$$

In the above, k is the dissociation constant of the acid. The concentration of the non-dissociated acid is equal to the "partial solubility" of the acid, which can be designated as λ, and transformed into the following equation:—

$$[\text{Acid anions}] = \frac{k \, . \, \lambda}{[H^{\cdot}]}.$$

The "total solubility" of the acid can be given the capital letter Λ, and the equation will now run

$$\Lambda = \lambda + \frac{k\lambda}{[H^{\cdot}]} = \lambda \, . \, \frac{[H^{\cdot}] + k}{[H^{\cdot}]}.$$

The "total solubility" Λ is therefore dependent upon the hydrogen ion concentration $[H^{\cdot}]$ of the solution, while the "partial solubility" λ is constant.

Applying this to the solubility of an amphoteric electrolyte it will be seen that the "total solubility" equals the "partial solubility" plus the concentration of the anions on the one hand and the kations on the other in a saturated solution of the ampholyte, the equation being as follows:—

$$\Lambda = \lambda + [A^{\cdot}] + [A'],$$

λ is always constant, therefore when Λ is a minimum $[A^{\cdot}] + [A']$ is a minimum, and this is the iso-electric point. Therefore an amphoteric electrolyte has its minimum solubility at that hydrogen ion concentration which corresponds to its iso-electric point. Every amphoteric electrolyte has two dissociation constants, an acid (k_a) and a base (k_b), which regulate the solubility.

From this it is clear that the lipoid-globulin particles in serum are kept in solution by the ions attached to them. It is also clear that the hydrogen ion concentration must be constant, otherwise the lipoid-protein molecules will begin to become precipitated, therefore however big or however ionic the lipoid-protein molecules become, the acidic and the basic ions will always be so arranged, that the hydrogen ion concentration's optimum will be maintained. This is the reason why the hydrogen ion concentration of a syphilitic serum is the same as that of a normal serum, although in each case the molecules themselves are different.

From the above it will be seen how intramine gets into solution *in corpore.* In virtue of its amino-groups it becomes adsorbed by the lipoid-globulin molecules. Since these molecules must maintain the hydrogen ion concentration's optimum, various electrolytes will be discharged, a preponderance of acidic over basic or *vice versâ,* according to the charge of the prevailing electrolyte in intramine. In the maintenance of this normal hydrogen ion concentration intramine gets into solution.

The greatest influence, then, of the hydrogen ion concentration will be that which it exerts upon the precipitation of colloidal solutions—an influence which has been frequently shown and alluded to in these lectures.

For the sake of clearness it will be as well to mention some of the important properties of colloids.

The one great distinction which all observers draw between a colloidal "solution" and a true solution is the difficulty with which the former and the ease with which the latter dialyses. Another and perhaps greater distinction is that a colloidal "solution" is really a suspension. A suspension is a micro-heterogeneous system, that is to say, a finely divided phase, the solubility of which in water is *nil*, which is suspended in an aqueous phase. Colloids which fulfil the above definition are known as suspension colloids or suspensoids, while proteins, which are in part soluble in water, are known as emulsion colloids or emulsoids. Emulsoids come midway between suspensoids and crystalloids. There is no hard and fast line between these three physical states, since emulsoids may, in some cases, be solutions of substances of high molar weight.

There are three great differences between emulsoids and suspensoids, and they are differences of density, viscosity, and surface tension.

Surface tension is the important one from our point of view. Speaking generally, the surface tension of suspensoids is the same as that of water, while the surface tension of emulsoids is usually much smaller than that of water. It is owing to this difference that suspensoids are not so readily adsorbed as emulsoids *in vivo*. The smaller the surface tension of a saturated solution the more this solution approaches a colloidal "solution." This property which, so to speak, stamps a colloidal "solution" consists in the great tendency the solid phase has, first, to remain suspended in high dispersity in the saturated solution, and secondly, when it is precipitated, for the precipitate to be amorphous. Hence the explanation for the peculiar properties witnessed in proteins. In the case of protein "solutions," which have a small surface tension, the ions attached to the solid particles play a very important *rôle*. As the solid particles are fully electrically charged, which keeps them in suspension *in corpore*, the surface tension vanishes and the particles remain still more firmly in suspension.

If the particles become in any way de-ionised the surface tension increases, and the small particles increase in size, with resulting agglutination and amorphous precipitation.

From these few remarks concerning surface tension, it will be readily seen why agglutination, complement-fixation, and precipitation are the pictures given by the immunity reactions *in vitro*; it will also be seen that all three are different phenomena of the same physical action, and that unless instantaneous death occurs, as is seen for instance in anaphylactic shock, none of these phenomena occurs *in vivo.*

The maintenance of this surface tension is dependent upon a certain hydrogen ion concentration. Therefore, the normal hydrogen ion concentration serves the purpose of keeping the lipoid-globulin colloidal particles in " solution " in the serum.

Now, as to how this normal hydrogen ion concentration is maintained.

The lipoid-globulin particles form the internal phase and the serum in which they are forms the external phase, therefore the normal hydrogen ion concentration will be maintained on the one hand by the external phase and on the other by the internal phase; although the action of each will be discussed separately, the two actions are of course interdependent.

There are what may be called two systems, which maintain the normal hydrogen ion concentration of the external phase: (1) the phosphate system; (2) the bicarbonate system.

The phosphate system is made up of an acidic and a basic sodium phosphate, the former being the mono-sodium phosphate (NaH_2PO_4) and the latter the di-sodium phosphate (Na_2HPO_4).

Like water these two salts are continually undergoing electrolytic dissociation as in the following equations:—

$$NaH_2PO_4 = Na^{\cdot} + H^{\cdot} + HPO_4''$$

$$Na_2HPO_4 = Na^{\cdot} + Na^{\cdot} + HPO_4''$$

$$H_2O \leftrightarrows H^{\cdot} + OH'.$$

$$H_2O + HPO_4'' = H_2PO_4' + OH'.$$

The bicarbonate system is very similar, as the following equations show:—

$$NaHCO_3 = Na^{\cdot} + HCO_3',$$

$$H_2CO_3 = H^{\cdot} + HCO_3',$$

$$H_2O \leftrightarrows H^{\cdot} + OH',$$

$$H_2O + HCO_3 = H_2CO_3 + OH'.$$

In this way a balance is maintained between the hydrogen ion and hydroxyl ion concentration of the external phase.

From the equations just given the number of hydrogen ions formed is the same as the number of hydroxyl ions, but, as I have already said, there are more hydroxyl than hydrogen ions in the serum. How then is this excess formed? Cells have functions to perform, for the performance of which they must have active oxygen. After the oxygen has been used up, it is discharged as carbon di-oxide. The presence of carbon di-oxide in the serum increases the concentration of the hydrogen bicarbonate (HCO_3'), this hydrogen bicarbonate undergoes electrical dissociation with water into hydrogen carbonate (H_2CO_3) and hydroxyl ions (OH'), thus giving rise to an excess of hydroxyl ions in the serum.

The normal hydrogen ion concentration of the internal phase is maintained by the oxidase-reducase system, which I will now repeat, as it is the chief link in my chain of research.

The oxidation process, or better to call it the oxidase system, has been well worked out by Bach and Chodat[2]. These two observers found that active oxygen was produced by the action of a ferment, which they called a peroxidase, upon a peroxide (hydrogen peroxide, H_2O_2). These two authors found furthermore that the peroxidase was a metal in the form of an hydroxide.

So far as active hydrogen was concerned, all we knew was that an extract of liver, which went by the name of Schardinger's[3] enzyme, would in the presence of an aldehyde form active hydrogen, but although Heffter[4] made the suggestion that Schardinger's enzyme was a sulphydryl-group, neither he nor anyone else has shown how this sulphydryl-group works, or what takes the place of the aldehyde in the body.

Owing to the invention of the word peroxidase, Schardinger's enzyme came to be called perhydridase, and by further analogy this perhydridase was stated to form active hydrogen by its action upon a perhydride (oxygen perhydride OH_2 or $H + OH$). From my own investigations, I am of the opinion that active oxygen is normally formed in the body by the action of ferric hydroxide-protein upon five atoms of hydrogen and two atoms of hydrogen peroxide, as in this equation

$$R.Fe(OH)_3 + 5H + 2H_2O_2 = R.Fe + 6H_2O + O''.$$

The hydrogen peroxide in the above equation is in my opinion formed, along with active hydrogen, by the action of di-sulphide-protein upon two atoms of hydrogen and four hydroxyl atoms, as in this equation

$$R.S.S.R. + 2H + 4OH = R.S + R.SH + 2H_2O_2 + H^{\cdot}.$$

The parasitic lipoid-protein particles, as well as the lipoid-protein particles in the serum of the host, since the host's resisting substance is always as nearly physico-chemically the same as that of the parasite, are now seen to possess a perfect oxidase-reducase system owing to adsorbed ferric hydroxide and di-sulphide ions, which on the one hand form active oxygen (O'') and on the other active hydrogen ($H^{\cdot}$). The great difference between the parasitic lipoid-protein particles and those of the host consists in the greater reducing action of the former, the significance of which will be seen later.

As the ferric hydroxide-protein and the di-sulphide-protein form active oxygen on the one hand and active hydrogen on the other hand, with the free hydroxyl and hydrogen ions formed by the electrical dissociation of the phosphate and bicarbonate systems with water, it will be seen that the maintenance of the hydrogen ion concentration of both the external and the internal phases is interdependent.

Since there is always a certain relationship between the hydrogen and hydroxyl ion concentration of the serum, a relationship which keeps the lipoid-globulin particles in "solution," it will follow that any alteration of the normal hydrogen ion concentration will result in the precipitation of those particles.

Since furthermore the lipoid-globulin particles are ampholytic it will follow that precipitation of the particles by a positive sol will be opposed by the hydroxyl ions, and that precipitation by a negative sol will be opposed by the hydrogen ions, hence the body has a perfect means of preventing precipitation of the internal phase in the external phase.

If the charge of the precipitating sol exerts its influence before the stabilising action of the serum is allowed full play, the normal hydrogen ion concentration may be so upset that death results. Such a phenomenon is met with in a condition known as anaphylactic shock.

Aluminium hydroxide, ferric hydroxide, and colloidal silicic acid when injected intravenously into rabbits cause almost instantaneous death. The two former are positive sols, while colloidal silicic acid is a negative sol. From what has just been stated the sequence of events following intravenous administration of these two different sols will be clear.

The positive sol will adsorb the hydroxyl ions as they approach to prevent precipitation of the particles by the positive sol, with the result that there will be an excess of hydrogen ions in the serum. The reverse will naturally be the case with the negative sol.

Aluminium hydroxide, then, increases the hydrogen ion concentration.

With the information thus gained the influence of aluminium hydroxide on staining and its action as a mordant become plain.

Methylene blue is an ampholytic dye, the acid moiety being the methylene red dye, and the basic moiety methylene violet. The methylene red being an acid dye will adsorb hydrogen ions, while the methylene violet will adsorb hydroxyl ions. If a weak base like sodium biborate is added it will increase the action of the methylene red, since it will adsorb hydroxyl ions and leave an excess of hydrogen ions. If a strong base like aluminium hydroxide is added it will adsorb hydrogen ions, leave an excess of hydroxyl ions, and increase the action of methylene red, but it quickly converts the excess of the negative hydroxyl ions into positive hydrogen ions, as the metal becomes free, with the result that all the methylene red disappears and only the methylene violet remains. The methylene violet itself then becomes partly decolourised owing to there being an excess of hydrogen ions. This explains why I was unable to substitute a better base for sodium biborate when I wanted to increase the action of the methylene red so as to get good vital staining of those phases of the *Leucocytozoon syphilidis* which showed such an affinity for this dye.

Mordanting is only another expression for the same phenomena which have just been mentioned. Cotton in water has a negative charge, and therefore will not stain with acid dyes. When cotton is put into a bath of aluminium acetate, the aluminium salt becomes hydrolysed into aluminium hydroxide sol and acetic acid. Adsorption now readily takes place between the negatively charged cotton and the positively charged aluminium hydroxide, with the result that a layer of the aluminium sol is deposited upon the cotton. The surface of the cotton now has a positive charge, in virtue of which it will readily adsorb acid dyes.

Anaphylaxis produced by proteins cannot be explained on the lines just followed, first, because the injected proteins are ampholytically charged; secondly, the charge is not in comparison so pronounced; thirdly, only the second or subsequent injections can produce anaphylaxis.

The symptoms of anaphylaxis are, of course, due to an upset of the normal hydrogen ion concentration, but this upset is caused indirectly. The first change which takes place when foreign proteins are injected is adsorption, an action which results in damaging the emulsoid character of the lipoid-protein particles in the serum. Owing to the attempt on the part of the lipoid-protein particles in the serum to bring back the particles of the injected lipoid-protein molecules to their perfect emulsoid state, they become in part de-ionised.

This de-ionisation destroys the oxidase-reducase system of the internal phase, and hence the normal hydrogen ion concentration of the external phase. Such a change increases the size of the lipoid-protein particles, makes them approach nearer to the suspensoid state, or to their iso-electric point.

From all that has gone before the nature of that mythical substance—complement—now becomes clear.

The observation of Michaelis and Skwirsky,[5] who found that red blood corpuscles which had adsorbed amboceptor, and which had been treated with mono- and di-sodium phosphate instead of with complement, only fixed the so-called middle piece of complement when it was added, leaving the end piece free to be collected if required, strongly supports my views as to the being and action of complement.

Complement is, in my opinion, the balance between the hydrogen ion and hydroxyl ion concentration of the serum, and some of the salts which regulate this balance are the phosphates of sodium.

The mono-sodium phosphate (NaH_2PO_4) behaves as a very weak acid, while the di-sodium phosphate (Na_2HPO_4) behaves as a very weak base.

The electrical dissociation of these salts is continually going on in the serum, as is also the electrical dissociation of the water.

The equations are as follows :—

$$NaH_2PO_4 = Na^{\cdot} + H^{\cdot} + HPO_4'',$$
$$Na_2HPO_4 = Na^{\cdot} + Na^{\cdot} + HPO_4''.$$

The electrical dissociation of water can be shown as follows :—

$$H_2O \rightleftarrows H^{\cdot} + OH'.$$

The combined electrical dissociation of the $H_2O + HPO_4$ ions can be shown as follows :—

$$HPO_4'' + H_2O \rightleftarrows H_2PO_4' + OH'.$$

In this way a balance is maintained between the hydrogen ion and the hydroxyl ion concentration of the serum.

A similar system to this phosphate system is likewise afforded by the bicarbonates.

$$\begin{aligned} NaHCO_3 &= Na^{\cdot} + HCO_3'. \\ H_2CO_3 &= H^{\cdot} + HCO_3'. \\ HCO_3' + H_2O &\rightleftarrows H_2CO_3 + OH'. \end{aligned}$$

Passing CO_2 through serum increases the concentration of the HCO_3' ion, hence an increase of the hydroxyl ion concentration. This results in the precipitation of the lipoid-protein molecules, since electrical dissociation of the ions at their surface will occur to neutralise this excess of hydroxyl ion. Neither the supernatant fluid nor the precipitated protein will act as complement, but if the precipitated protein is collected and washed well with sodium chloride, complement action is regained, since sodium chloride in water neutralises the excess of the hydroxyl ion by the liberation of a positive ion, hence restoring the equilibrium between the hydrogen ions and hydroxyl ions. The middle piece of complement is the globulin portion, and this is the piece which is fixed in Michaelis and Skwirsky's experiments when amboceptor and red blood corpuscles are treated first with mono- and di-sodium phosphate. This piece is fixed because of the amino-groups it contains and because the phosphate system, establishing an equilibrium as it does between the hydroxyl ion and the hydrogen ion concentration of the serum, allows the middle piece to be adsorbed. This proves to my mind that the phosphate system has taken the place of the so-called end piece, and as in these experiments the end piece can be collected afterwards intact, the presumption may be made that the end piece is that which keeps the hydrogen ion concentration of the fluid part of the serum constant. The middle piece will then be that which keeps the hydrogen ion concentration of the solid part (lipoid-globulin molecules) of the serum constant, which is the oxidase-reducase system. From this it follows that complement is the same as the catalysts hydrogen ($H^{\cdot}$), hydroxyl (OH'), the action of which is to keep the lipoid-globulin particles in "solution" in the serum. If the equilibrium is destroyed between these two catalysts, the lipoid-globulin particles cease to be in "solution," as they are made to approach their iso-electric point, at which they are precipitated. Agglutination is a pre-stage of precipitation and precipitation results in an alteration of surface tension. Both agglutination and precipitation destroy complement action since they upset the normal hydrogen ion concentration. Therefore immunity reactions *in vitro* are, when specific, chemico-physical, when non-specific, purely physical reactions, in which the lipoid-globulin particles are made to approach to their iso-electric point owing to the adsorption between incompletely charged particles, which, in their attempt to become equally charged, destroy the normal hydrogen ion concentration. One more point in favour of this view is that fresh specific serum will sometimes fail to agglutinate, active syphilitic serum will sometimes fail to give a positive Wassermann reaction, active specific serum when mixed with fresh antigen will sometimes fail to fix complement in a bacterial complement-fixation test, and precipitin reactions will sometimes fail to be positive, when the ingredients used are absolutely fresh, because the colloidal particles are completely charged and, therefore, the equilibrium between the hydrogen ion and hydroxyl ion concentration of the serum is not upset.

In nothing is the importance of the hydrogen and hydroxyl ions so clearly shown as in chemotherapy. In the chapter on chemotherapy I attempted to show that the action was mainly one of oxidation and reduction, metals in the form of hydroxides acting as oxidising agents, and a non-metal in the form of a di-sulphide acting as the reducing agent.

Basic hydroxides of metals migrate to the kathode, and, being metallic hydroxides, they can form metallic (positive) ions by electrolytic dissociation, and at the same time produce an equivalent amount of hydroxyl ions (OH').

Sulphides migrate to the anode, and, by electrolytic dissociation, they can form non-metallic (negative) ions (sulphur), and at the same time produce an equivalent amount of hydrogen ion ($H^{\cdot}$).

From this it can be inferred that iron hydroxide, the hydroxide that normally exists as the oxidising agent, arsenic hydroxide, and aluminium hydroxide, the last two hydroxides acting as extra oxidising agents in chemotherapy, adsorb hydrogen ions ($H^{\cdot}$) more readily than hydroxyl ions (OH').

Sulphides will on the same principle adsorb hydroxyl ions (OH') more readily than hydrogen ions ($H^{\cdot}$).

The reduction process, in my opinion, goes as follows: Sulphur is the chief agent, and therefore a description as to how sulphur works normally in the body will also apply to the sulphur which aids the action when intramine is used.

The sulphur forms a di-sulphide-protein, which adsorbs two hydrogen and four hydroxyl atoms, to form sulphur-protein, a sulphydryl- or mercaptan-protein, two atoms of hydrogen peroxide, and one hydrogen atom, which is the active hydrogen.

The mercaptan-protein adsorbs free sulphur, to form di-sulphide-protein and hydrogen sulphide,

$$2R.SH + S = H_2S + R.S.S.R.$$

$$R.S.S.R + 2H + 4OH = R.S + R.SH + 2H_2O_2 + H^{\cdot}.$$

Under certain circumstances the mercaptan-protein seems capable of adsorbing free oxygen, to form di-sulphide-protein and water,

$$2R.SH + O = R.S.S.R + H_2O.$$

The oxidation process, in my opinion, goes as follows: Iron hydroxide-protein adsorbs five hydrogen atoms and two hydrogen peroxide atoms, to form iron-protein, six atoms of water, and active oxygen.

The free iron then forms an hydroxide. The action of arsenic in salvarsan, and of aluminium in aluvine, and of iron in ferrivine, doubtless run a similar course to that of the iron hydroxide-protein, which normally exists in the body

$$R.Fe + 4H_2O = R.Fe(OH)_3 + 4H + OH.$$

$$R.Fe(OH)_3 + 5H + 2H_2O_2 = R.Fe + 6H_2O + O''.$$

From these equations it is clear that the hydrogen and hydroxyl ions, the ions upon which the chemical processes are dependent, are themselves not stoicheiometrically concerned, a proof that the hydrogen and hydroxyl ions are catalysts.

The classical example of a catalytic action is the inversion of saccharose by acids. Through the work of Arrhenius[6 7] we know that the rate at which the inversion occurs is proportional to the hydrogen ion concentration.

The chief action in the body is an interchange between oxygen and carbon di-oxide. Oxygen is the basis of most of the actions in the body; it is by means of oxidation that parasites become destroyed.

Fever, one of the body's greatest protective mechanisms, means an increase of the hydroxyl ion concentration, and therefore more oxygen. Note, further, Loeb's[8 9] work on the influence of the hydroxyl ions on artificial parthenogenesis. Reduction is only a means of increasing oxidation as it forms the peroxide necessary for the oxidase system. In view of these facts it is not to be wondered at that the serum is normally on the alkaline side of neutrality, in other words, that it possesses a preponderance of hydroxyl over hydrogen ions. As I have already stated oxidation is more to the fore in early syphilis, and reduction is more to the fore in late syphilis. This means that in late syphilis all efforts are being put forward to produce the oxidation power necesssary to overcome the parasite, and this is done indirectly by increasing the reducing power of the lipoid-protein molecules.

When the patient first gets syphilis and his lipoid-protein particles are, so to speak, new at the game, their power of oxidation will be at its prime, therefore they will not employ indirect methods to increase such power, consequently any attempt to increase their reducing power would be expected to produce an effect exactly contrary to that hoped for. Such an effect can be produced by prescribing intramine before a metallic compound has been used. It seems to me to be in these cases that the mercaptan-protein adsorbs the free oxygen.

The only means which might be expected to accelerate or aid the oxidation power of the lipoid-globulin particles would be those which act directly in increasing the oxygen ions [O'']. From this it follows that metals, such as iron, aluminium, and arsenic in their tri-valent forms, in virtue of their action in adsorbing more hydroxyl than hydrogen ions, are the best means of getting rid of the parasite in early syphilis, an action with which we are well familiar.

If what I have said concerning reduction is true, it may be expected that intramine given before salvarsan in early syphilis would not be nearly so efficacious as administering salvarsan first and intramine afterwards, and that in some cases, by giving intramine first, the symptoms might even be aggravated. In practice this is exactly what happens, owing as I have already explained, to the mercaptan-protein adsorbing free oxygen.

The administration of salvarsan, aluvine, and ferrivine, acting as they do as extra peroxidases to the iron hydroxide already present, uses up all the available hydroxyl ions; it is therefore necessary to find indirect means of producing more, such as by increasing the reducing action of the lipoid-protein molecules, which act in virtue of their di-sulphide-groups (perhydridase), The reducing action can be increased by intramine (sulphur), therefore the alternate administration of a strongly adsorbed metallic compound and intramine would be expected to produce the maximum beneficial effect possible in early syphilis. Once again, in practice this is exactly what happens. Arguing on the same lines, only reversely, it will at once be seen that in recurrent and late syphilis, the administration of intramine should precede that of the strongly adsorbed metallic compounds.

Another important action dependent upon the hydrogen ion concentration, although not directly concerned with the subject in hand, is of sufficient interest to warrant insertion here—I am referring to phagocytosis. Indirectly, it has a bearing upon the subject in hand, since from the influence the hydrogen ion concentration has upon phagocytosis, the means by which the *Leucocytozoon syphilidis* can live and propagate in the human host may be inferred.

Hamburger and Hekma[10] showed that a diminution or an increase of the hydroxyl ion concentration of the serum caused a fall in the phagocytic power of the polymorphonuclear leucocytes. They showed further that calcium ions increased the phagocytic power, and that quinine very seriously diminished it.

This remark about quinine is interesting, as it confirms what I have always maintained, namely, that phagocytosis plays no part in the combatting of protozoal diseases.

The reason why calcium ions act beneficially is, doubtless, due to their power in increasing the stability of the permeability of the leucocytes, thereby enabling them to take advantage of the hydroxyl ions they already possess, without being influenced by a change that is taking place in the external phase.

Before drawing further inferences from the paper just mentioned, it will be as well to refer to another interesting paper on the influence of the hydrogen ion concentration on the movements of the amœba by Hirschfeld.[11]

This observer found that a diminution of the surface tension occurred at that spot where the amœba threw out a pseudopodium.

The moment this pseudopodium touched the object it desired to attract to itself, a local rise of the surface tension occurred, contraction followed, and the object was drawn in with it.

The amœba can be regarded as an ampholytic body, therefore any change in the hydrogen ion concentration of the fluid medium in which it is, will influence the movements thereof. The same will be the case with a polymorphonuclear leucocyte.

The reader will now see why serum should be on the alkaline side of neutrality, since there are more hydroxyl than hydrogen ions, and why fever should be beneficial in disease, since it increases this alkalinity.

Hirschfeld found that the increase of the hydrogen ion concentration in a bacterial suspension caused, when some of the bacteria were placed by amœbæ, a lowering of the surface tension of the protozoa, with the result that the protozoa threw out pseudopodia and ingested the bacteria.

Hirschfeld found also that if amœbæ were injured, a rise in the surface tension occurred, which resulted in a diminution of the electric charge, and the carbon di-oxide cloak, so to speak, formed by exhalation vanished.

Should the change go so far, that the amount of carbon di-oxide exhaled falls to *nil*, the electric charge entirely disappears and coagulation of the protozoa sets in.

It is highly probable that this diminution of the electric charge on the surface of bacteria causes them to form capsules and spores and stimulates them to take on an intra-cellular habitat. Diseases caused by organisms which are capsulated, or form spores, or have an intra-cellular life are, as is well known, extraordinary difficult to cure. Taking an infection with which I am most familiar—namely *Ulcus molle*. *Ulcus molle* is due to a strepto-bacillus which is not capsulated, nor does it form spores, and it is extra-cellular. A complication of this disease, a chronic ulceration, known as *Ulcus molle serpiginosum*, is due to the same strepto-bacillus, which has taken on an intra-cellular habitat. Ordinary *Ulcus molle* is easy to cure, while the chronic ulceration is most difficult to cure.

The higher in the scale of evolution an organism becomes the greater seems its tendency to either form spores or to live an intra-cellular life. Some bacilli form spores, all fungi do, and most pathogenic protozoa pass some part of their existence in the cells of the host they attack.

From these few remarks on protection the explanation of natural and acquired immunity becomes clear. To take one example of natural immunity. Lizards under ordinary circumstances are immune to tetanus, but if their temperature be raised they can be infected. We know that alterations of temperature influence or change the hydrogen ion concentration; we know further that a change in the hydrogen ion concentration means an alteration of the surface tension of the protective substances, and that an alteration of the surface tension is concomitant with a change in the colloidal state of the internal phase. If these changes occur unnaturally, as they certainly would do if the temperature of a cold-blooded animal were raised, it might reasonably be assumed that the changes would act adversely, and so favour an infection, which under normal circumstances would not have occurred. From this it can be seen that immunity may readily be influenced by the number of organisms implanted upon the host, and by the position where they are implanted.

So far as acquired immunity is concerned, syphilis will be taken as the example. When a man runs the risk of becoming infected with syphilis, there must always be, first of all, a struggle between the cells of that man and the spores of the *Leucocytozoon syphilidis*. If the former win, then, of course, there is no infection. If the latter win there is an infection. The spores having won, they will develop at their host's expense and produce a lesion, which we call a chancre. A further development of these spores will lead to a generalisation of them in the body of the host, with a result that lesions will occur all over the body. Instead of the development going further still, the tide turns in favour of the host and the number of the parasites gradually becomes lessened, with the result that the lesions disappear. The turning of the tide is due to the fact that the host's lymphocytes have been able to manufacture lipoid-globulin particles, the molecular configuration of which is homologous to that of the lipoid-globulin molecules of the parasite.

Two questions now arise: (1) What is the nature of the primary struggle? (2) What is the *modus operandi* of the turning of the tide?

From the remarks I have made already it will be remembered that there are only two factors which come into play in the adsorption between antigen and antibody; (1) hydroxyl ion concentration (oxidase-reducase system), (2) specific amino-groups.

In the primary struggle the second factor cannot possibly play a *rôle,* since it takes some time for the amino-groups in the lipoid-globulin molecules to be arranged in a specific manner; moreover, the primary struggle is a local affair, a struggle between spore and cell, while the turning of the tide is a general affair, a struggle between the lipoid-globulin particles of the serum generally, of the plasma cells locally, and those of the parasites.

When a syphilitic spore enters the human body, to develop it must enter a connective-tissue cell or an endothelial cell. To enter a cell it must alter the surface tension of that part of the cell where it is going to enter, and this will mean a local disturbance of the normal hydrogen ion concentration. It is certain that most cells require and use oxygen, and that most cells in exchange for oxygen exhale carbon di-oxide. Should a spore be able to take some of the oxygen required by the cell it hopes to enter, the ratio between the oxygen and the carbon di-oxide of that cell will cease to exist, with the result that the normal hydrogen ion concentration, or the oxidase-reducase system, will be locally temporarily deranged, with the result that the spore wins, since the local surface tension of the cell will be such that the spore is easily able to enter it. How this temporary derangement of the oxidase-reducase system can best be directly prevented is not at present absolutely certain, but from analogy to experiments I have undertaken in another direction (anaphylaxis), the derangement can be prevented indirectly by increasing the stability of the permeability, which can be done by intravenous injections of calcium chloride. We know that alcohol increases the permeability of cells, and also that patients are more liable to contract syphilis, when under its influence. Therefore, it is quite reasonable to think that a salt-rich diet, or an injection of calcium chloride before connection, would considerably diminish the patient's likelihood of becoming infected.

The *modus operandi* of the turning of the tide is probably as follows :—

Both the lipoid-globulin particles of the syphilitic phases, and of the serum, have an oxidase-reducase system. In the case of the former, the active oxygen is used by the nucleus for propagation purposes. In the case of the latter, there being no nucleus, the active oxygen will be used for that purpose for which the particles exist, namely, for the purpose of adsorption. Since the amino-groups have the same arrangement in the two kinds of lipoid-globulin particles, the necessaries for the physical action of adsorption will be at their best. As the active oxygen in the host's lipoid-globulin molecules will only be required for this physical action, it will follow that the host's lipoid-globulin molecules have an advantage over those of the parasite, with the result that the changes which the spore in the primary struggle caused the cell it was attacking to undergo, will now be visited upon the parasites themselves. If these views are correct, we should be able to forestall the turning of the tide and increase it, by increasing the amount of active oxygen in the lipoid-globulin particles in the serum of the host. This can be done directly by (1) the administration of strongly adsorbed metallic compounds, which act as peroxidases; (2) the administration of a strongly adsorbed non-metallic compound, which acts as a di-sulphide.

The examples showing the importance of the hydrogen and hydroxyl ions in biology could be multiplied almost without measure, since the " solid " particles in the serum or the host's resisting substance are kept in " solution " by these ions.

A final remark on a characteristic feature of emulsoids—the colloid group, which embraces proteins—will form a fitting end to this chapter.

Emulsoids are hydrophilic, that is to say, the disperse or internal phase shows an affinity for, and contains in its particles—water. It is the presence of this water which renders the internal phase "liquid" in the case of emulsoids, and any abstraction of this water tends to convert emulsoids into suspensoids.

Suspensoids are not hydrophilic, the particles which form the internal phase do not contain water, hence the internal phase is practically a solid.

The adsorptive capacity of suspensoids is not equal to that of emulsoids, hence proteins in the body must be emulsoids, as otherwise they would be unable to form additive compounds or render "soluble" substances which, under ordinary circumstances, are insoluble.

Any change which deprives emulsoids of the water they contain, such as boiling, denaturation, salting out, etc., converts them into suspensoids, in which state they cannot fulfil their proper function.

Since water is a mixture of hydrogen and hydroxyl ions, and since it is due to these ions that the lipoid-globulin molecules in the serum are emulsoids, and are kept in "solution," it is immediately obvious that any change in the hydrogen ion concentration will affect the water content of the internal phase, and tend to convert the particles from an emulsoid into a suspensoid state, in which condition their function is impaired. It was from the observance of these simple facts, that the E-S. tests sprang into being.

REFERENCES.

[1] Michaelis (1913), "Handbuch der Biochemie," Ergänzungsband. Fischer, Jena.

[2] Bach u. Chodat (1903), "Berichte der deutsch. chem. Gesell.," xxxvi, 600.

[3] Schardinger (1902), "Zeitschr. f. Unters. Nahrungs. u. Genussmittel," v, 1113.

[4] Heffter (1908), "Arch. f. exper. Pathologie," Festschr. f. Schmiedeberg, 253.

[5] Michaelis u. Skwirsky (1910), "Zeitschr. f. Immunitätsforschung," iv, 357, 629.

[6] Arrhenius (1907), "Immunochemistry." Macmillan, London.

[7] Arrhenius (1912), "Conférences sur chimie physique." Hermann, Paris.

[8] Loeb (1900), "Amer. Journ. of Physiol," iii, 135.

[9] Loeb (1909), "Die chemische Entwicklungserregung des tierischen Eies." Springer, Berlin.

[10] Hamburger u. Hekma (1908), "Biochem. Zeitschr.," ix, 273, 512.

[11] Hirschfeld (1909), "Zeitschr. f. allgem. Physiol.," ix, 529.

SUMMARY.

The cause of syphilis is a protozoon belonging to the order *Coccidiidea*, and the species which most befits it is the *Leucocytozoon*, hence my name for the parasite—*Leucocytozoon syphilidis.*

The *Spirochæta pallida* is the adult male phase, and it may be chemically recognised by its powerful reducing action, which appears to be due to its containing an unsaturated fatty acid in its molecule. This unsaturated fatty acid renders the permeability and adsorptive powers of its "membrane" very sensitive, with the result that it is readily stained with silver, and easily killed by therapeutic means, which are metallic in nature, in virtue of the positive charge of their ions.

The *Spirochæta pallida* imparts this increase of sensibility to the female cell during impregnation.

The other phases also have a strong reducing action, stronger indeed than that exhibited by nucleoli and by the protoplasm of plasma cells, but not so pronounced as in the two phases just mentioned.

The reducing action is due to the presence of active hydrogen, which is formed by the action of di-sulphide-protein on free hydrogen and hydroxyl ions. The reducing action is recognised by the affinity the lipoid-globulin molecules exhibit for pyronin and by the readiness with which they decolourise methylene violet, methyl green, and malachite green.

Owing to the strong oxidising action of nuclei, due to the presence of active oxygen, which is formed by the action of ferric hydroxide-protein on free hydrogen and hydroxyl ions, they stain readily with methylene violet, etc., but decolourise pyronin.

The reason why the *Leucocytozoon syphilidis* does not stain with methyl green, when pyronin is present, is not because there are no nuclei, but because staining is only a surface condensation, and because the reducing action on the surface is stronger than the oxidising action.

The phases of the *Leucocytozoon syphilidis* do contain nuclei, and nuclei which possess particularly strong oxidising properties.

The arrangement of nucleoli is similar to that met with in parasites, *i.e.*, strong reducing lipoid-globulin molecules cover, so to speak, underlying strong oxidising nuclein molecules. The nucleolus is the most vital part of the cell, the part where oxidation and reduction go most hand in hand.

The active oxygen obtained indirectly by reduction, and directly by oxidation, in the case of the nucleolus and the parasite, is used by the nuclein for propagation.

The active oxygen, in the case of the protoplasm of the plasma cells, is used partly as the activator of the adsorptive action performed by its lipoid-protein molecules.

The plasma cell originates from the lymphocyte, and forms lipoid-protein particles, which, with those of the serum, form the resisting substance.

This resisting substance is the basis of immunity.

Immunity, including anaphylaxis, staining, and chemotherapy are colloidal actions, and, in the main points, all are the same, since adsorption, oxidation, and reduction play an important part in each.

Immunity can be divided into groups: (1) natural; (2) artificial.

Natural immunity is due to certain physical conditions under which the resisting substance, or the lipoid-protein particles of the serum, exist.

Artificial immunity may result from a bygone attack of the infection in question, or may be produced by giving the infection in some attenuated form (smallpox), or by forcing the production of antibodies by injecting bacteria, alive or dead, etc.

Natural immunity shows no specificity, but artificial immunity does.

The resisting substance is produced mainly by the lymphocytes, from which it is excreted into the blood stream.

The resisting substance must be regarded as particles, therefore they have a surface, forming emulsion colloids in a fluid medium, which mainly consists of water.

Each particle must be looked upon, not as one big molecule, but as made up of an infinite number of complexes.

The substance to be attacked is constituted similarly to the resisting substance.

The dual substances (resisting and parasitic) are electrically charged, and the charge is accumulated at the surface (Faraday's law).

As both emulsion colloids exist in a fluid medium, there will be continual electrolytic dissociation at the surfaces.

As is well known, the charge on the surface of a solid in a liquid implies the existence of an equal and opposite one on the corresponding surface of the liquid.

By upsetting this balance, as it would be theoretically possible to do, the resisting substance could vanquish the parasitic substance.

If such was the case, it would mean a deposition or concentration of particles from the resisting substance on the surface of the parasitic substance, and decreasing the free energy present on the surface of the latter, according to the "Gibbs-Thomson" law, such a process being called "adsorption."

Such a process can kill the organisms, without effecting any microscopic change in the latter. The organism probably then undergoes auto-oxidation.

Such a process as this can be regarded as one of electrochemical neutralisation, and one which occurs in natural immunity.

In artificial immunity, which is specific, the same process may occur, but it is difficult to explain at present the exact way in which the amino-groups act, as it is upon these groups that the phenomenon of specificity depends. The most reasonable explanation to offer at present is that the amino-groups of the resisting and parasitic substances have, in each individual case, homologous stereo-chemical molecular configurations. If correct, this would imply that both substances had the same electro-chemical affinities, but as the results of the electrical dissociation in both cases are different it would allow for the concentration of particles of the resisting substance upon the surface of the parasitic substance, thereby lowering the surface tension, and altering the permeability of the latter, so causing its death.

The two main reasons for supposing that the resisting substance possesses an homologous stereo-chemical molecular configuration to that of each parasite it attacks, are (1) that any interference with the amino-groups destroys specificity, (2) that it takes some time for the resisting substance to get itself into that condition in which it is able to combat the infection.

During this preparatory period, the parasites become generalised in the system and can more or less do as they like, hence we have the explanation of the generalisation stage of syphilis.

For adsorption to produce more than a mere surface change, it is necessary that the molecules concerned in the process contain active oxygen. Active oxygen is formed directly at first and indirectly afterwards. Active oxygen is formed directly by peroxidases, which are hydroxides of metals, and indirectly by a perhydridase, which is a di-sulphide-protein.

The di-sulphide-protein forms hydrogen peroxide and active hydrogen. The hydrogen peroxide is converted into active oxygen by ferric hydroxide-protein.

Active oxygen is required not only by the lipoid-globulin molecules of the serum and of the plasma cells, but by the syphilitic parasites as well. As the active oxygen in the case of the first two is used in increasing their attacking power, and in the case of the parasite solely for propagating purposes, it might be assumed that the advantage would be with the former, which it is; hence the death of the parasites, which takes place, in time, without any outside assistance.

When extra pressure is put upon the resisting substance to combat the parasites, it increases its reducing action, and it does so by increasing first its globulin and then its "lipoid" content; hence the reason for the greater preponderance of the carboxyl-groups in the resisting substance in the later stages of chronic infections. There is probably only one protein—albumin; this by increasing the number of its amino-groups becomes globulin, by increasing its carboxyl-groups it becomes lipoid-globulin. An increase of carboxyl-groups results not only in making the reducing action greater, but also in increasing the size of the particle and rendering it more permeable, which results in that particle becoming less ionised. The change may be so great that the particle, or rather the cell in which the particle is manufactured, drops its relationship with its fellow cells and becomes a foreign body, so to speak.

Such a changed cell behaves like a parasite, and when this is the case the host develops malignant disease. The first ions to vanish when the lipoid-protein particles become more "lipoidal" are the calcium ions, hence the reason why, in the late cases of syphilis, the clotting time of blood is not so short as it is in the early cases, and why the patches of syphilitic atheroma contain no calcareous plates (Andrewes). The disappearance of the calcium salts aids in increasing the permeability of the molecules.

Outside the body, the resisting substance is made to act in a different way, and, as observers have noticed, there were many more ways than one, so it was supposed that there was more than one resisting substance. The resisting substance is a colloid, and the characteristic proporties of colloids are numerous, and such tests as agglutination, precipitation, complement-fixation, etc., are only exhibitions of these properties.

The fundamental principle of immunity tests is the same in all. The principle is again adsorption and electro-chemical neutralisation of the bodies adsorbed. *In vitro*, the adsorption is gross, a marked change in surface tension occurs, the change in permeability of the surfaces of the bodies adsorbed is great, and, according to the procedure adopted, the adsorption may show itself as an agglutination, a precipitation, or a lysis. None of these phenomena occur *in vivo*.

The nature of dyeing and staining is again one of adsorption, and the same peculiarities are to be noted as in immunity, in comparing staining *in vivo* with that *in vitro*. *In vivo*, cells stain badly and, broadly speaking, only basic dyes are suitable.

This is due to the fact that protein exists in a medium on the alkaline side of neutrality, which, although it is amphoteric, makes the negative greater than the positive charge.

It is probable that the dye particles, during life, never reach further than the surface of the object which they dye. As a result of adsorption, the surface soon becomes altered, because a proper balance cannot be maintained, since observations made with so-called *in vivo* staining are not strictly parallel to what would occur in the body. *In vivo* adsorption of the dye sooner or later results in the death of the cell, without producing any metamorphic change thereof, but the cell, as death approaches, gradually stains more deeply, and when death has occurred it stains well.

Staining dead or fixed material is analogous to the immunity tests, or immunity *in vitro.* The cells, owing to a loss of electrolytes which results at death, are far more susceptible to electrolytes than they are when *in vivo*, with the result that the adsorption changes are gross, in other words, a considerable portion of the dye enters the cell. Such a process is exactly analogous to agglutination, precipitation, and complement-fixation. In immunity, reduction and oxidation play important *rôles*, as they do in staining.

The normal hydrogen ion concentration is also an important factor in immunity reactions, and marked deviation from the concentration's optimum *in vivo* results in anaphylaxis. The hydrogen ion concentration of the external phase (fluid part of the serum) corresponds to the oxidase-reducase system of the internal phase (lipoid-globulin particles), but both are interdependent, as any injury to the one upsets the other. The most important function of the normal hydrogen ion concentration is the influence it exerts in making colloidal particles "soluble." Any disturbance of the concentration's optimum results in the particles being rendered less soluble. When colloidal particles are in "solution" they are emulsion colloids, but when they are thrown out of "solution" they become suspension colloids. The change from an emulsoid into a suspensoid is due to extraction of electrolytes from the emulsoid particles.

It follows that all the immunity reactions *in vitro* are only different phases of the one phenomenon—change in the colloid character of the particles concerned.

Adsorption between unequally charged particles, results in the particle which is more fully charged giving up some of its electrolytes to the particle which is less fully charged. If such a change takes place in a medium in which there is a normal hydrogen ion concentration and an oxidase-reducase system, adsorbed particles will attempt to return to their fully charged state, which they can only do at the expense of the fully charged particle, with its oxidase-reducase system existing in a medium with a normal hydrogen ion concentration. This produces a still further over-balance, with the result that the particles become still more suspensoid. There will naturally be a ratio between the degree of electrolytic dissociation and the degree of conversion from the emulsoid into the suspensoid state.

When slight, the change witnessed is only that of agglutination, if the change is more pronounced precipitation and complement-fixation occur, while if hydrolysis of the precipitated particles is allowed to ensue, a partial conversion of emulsoids into crystalloids follows. None of the changes mentioned occurs in the ordinary course of events *in vivo*, because the body is always able to maintain a normal hydrogen ion concentration in the serum, with the result that the emulsoid particles never normally become converted into suspensoids.

If the immediate disturbance of the normal hydrogen ion concentration is very great, the emulsoid particles may become partially converted into suspensoids, before the body is able to rectify the change; this results in sudden death, and is known as anaphylactic shock. Anaphylactic shock is usually produced by the intravenous administration of suspension colloids. Ordinary anaphylaxis and chronic anaphylaxis occur in the same way as has just been described, only the changes produced are less severe. Anaphylaxis can always be avoided if inorganic colloids are rendered emulsoid, and if the ions of organic emulsion colloids are prevented from undergoing electrolytic dissociation on the surface, before they are injected. Anaphylaxis, having once occurred, can be stopped by the administration of calcium chloride, which increases the stability of the permeability of the particles in "solution," thus saving half of the normal hydrogen ion concentration, *i.e.*, the oxidase-reducase system, from becoming upset.

In chemotherapy the same factors are at work. Salvarsan is an emulsion colloid, and is adsorbed by the syphilitic parasites, the protoplasm of the plasma cells, and the lipoid-globulin particles of the serum.

The reason why salvarsan appears to have a specific action is simply due to the size of the anti-protozoal colloidal particles and to their increased adsorptive action. Since parasites are killed by adsorption, an action which is dependent upon active oxygen, it will follow that the oxidising and reducing properties of the lipoid-globulin molecules will be more marked in syphilis and in other protozoal diseases, than in bacterial infections.

For adsorption between the lipoid-globulin molecules of the serum and the parasites to take place *in vivo*, it is necessary for the particles of both to have an homologous stereo-chemical molecular configuration. As the molecular configuration of no lipoid-globulin particle is known, still less when that particle is specific, it is perfectly clear that remedial agents cannot be manufactured to simulate such particles, as for instance vaccines do.

Specific adsorption, like most chemico-physical actions, is dependent upon the presence of active oxygen, which is formed directly in the early stages of the disease by adsorbed metallic hydroxides and indirectly in the late stages of the disease by an adsorbed di-sulphide.

Therefore we can manufacture remedial agents which will increase the amount of active oxygen directly and indirectly.

Metallic compounds act as oxidising agents and non-metallic compounds as reducing agents, therefore the arsenic in salvarsan acts catalytically as a peroxidase, and the sulphur in intramine acts catalytically as a perhydridase.

As arsenic is a toxic metal and does not simulate or increase the normal peroxidase, as does the sulphur in intramine, its place is better taken by iron, as in ferrivine. Ferrivine and intramine then increase the amount of active oxygen by the same means as the host himself employs, only in a greater degree.

For the iron and sulphur to act respectively as a peroxidase and perhydridase, it is necessary that they should be emulsion colloids. As emulsion colloids, when injected they are adsorbed by the lipoid-globulin molecules in virtue of the amino-groups their chemical molecules possess. When adsorption has occurred between the amino-groups, the iron and sulphur are able to form protein adsorption compounds, as only in such a state can they act as a peroxidase and perhydridase.

As every specific adsorption process is dependent upon the supply of active oxygen, the continued alternate administration of intramine and ferrivine will improve any chronic infection, but naturally the improvement produced will bear a corresponding ratio to the size of the protective particles of the host's resisting substance.

As the size of the particles of the resisting substance is always greater in the chronic stages of infections than in the acute stages, as also the permeability of the said particles is more marked in the chronic stages, the remedial action of intramine and ferrivine is correspondingly more pronounced.

In the acute stages of an infection, oxidising (and therefore metallic) compounds should precede reducing (and therefore non-metallic) compounds, while in the late stages the order should be reversed.

Iodine probably acts as a reducing agent. It increases the action of intramine as it prevents the mercaptan-protein from taking up oxygen.

Mercury, owing to its high atomic weight, acts mainly in increasing the adsorptive capacity of the colloidal lipoid-globulin particles, and possibly slightly as a peroxidase.

As the main difference between protozoal and bacterial diseases is one concerning the size of the lipoid-globulin molecules in the serum, it will follow, in protozoal diseases in which the lipoid-globulin molecules are biggest, that the molecules will differ as to their water content, and that the rate and ease with which the emulsoid particles can be converted into suspensoid particles will vary. Simple and rational tests can be devised to detect the variations, and tests which give an indication not only to the kind of disease from which the patient is suffering, but also to the state of the protective power the host is exhibiting in that disease. A simple syphilitic test is the E-S. test.

IMMUNITY ACTION *in vivo.*

Adsorption between the lipoid-protein particles of the resisting substance and the lipoid-protein particles of the parasite, in the presence of an oxidase-reducase system, and existing in a medium in which there is a normal hydrogen ion concentration. Such an action is specific, because an homology exists between the stereo-chemical molecular configuration of the amino-groups in the two lipoid-proteins. Change produced is an alteration of the permeability, with consequent autolysis of the lipoid-protein particles.

IMMUNITY ACTION *in vitro.*

(a) *Specific.*

Adsorption between the lipoid-protein particles of the resisting substance and the lipoid-protein particles of the parasite, in the presence of an oxidase-reducase system, and existing in a medium in which at first there is a normal hydrogen ion concentration. The action is specific, because an homology exists between the stereo-chemical molecular configuration of the amino-groups in the two lipoid-proteins. Change produced is an alteration of the surface tension, with precipitation of the bodies adsorbed, and the destruction of both the oxidase-reducase system of the internal phases and the normal hydrogen ion concentration of the external phase. The phenomena witnessed according to the degree of the change produced are, agglutination, precipitation, complement-fixation, and hydrolysis.

(b) *Non-Specific.*

Adsorption between the lipoid-protein particles of the resisting substance and an emulso-suspension colloid in the presence of an oxidase-reducase system, and existing in a medium in which, at first, there is a normal hydrogen ion concentration.

The action is more or less specific to protozoal diseases, especially syphilis, owing to the increased size and adsorptive capacity of the lipoid-protein particles of the resisting substance in these diseases. Change produced is an alteration in the surface tension, with precipitation of the bodies adsorbed, and the destruction of both the oxidase-reducase system of the internal phases and the normal hydrogen ion concentration of the external phase. The phenomena usually witnessed are complement-fixation and hydrolysis (Abderhalden's test).

ANAPHYLAXIS.

Adsorption between the lipoid-protein particles of the resisting substance and a suspension colloid, if the reaction is non-specific, and an emulso-suspension colloid if the reaction is specific, in the presence of an oxidase-reducase system, and existing in a medium in which there is, at first, a normal hydrogen ion concentration.

Change produced is an alteration of the surface tension—a change of the emulsoid particles into a partly suspensoid state, with the destruction of both the oxidase-reducase system of the internal phases and the normal hydrogen ion concentration of the external phase. Whether the anaphylaxis is acute enough to produce death, or is only chronic, depends upon the degree produced in the changes above mentioned.

STAINING.

Adsorption between the lipoid-protein particles and the colloidal particles of the dyes. Basic dyes are most easily adsorbed *in vivo*, owing to staining being, as all adsorption processes are, only a surface action, and to the fact that the adsorptive power of the active oxygen in the oxidase-reducase system is more marked than that of the active hydrogen, owing to the fact that active oxygen is di-valent and active hydrogen is mono-valent.

In the cases in which the lipoid-globulin particles adsorb acid dyes in preference, it is due to the strong reducing action of the particles, or, in other words, to the powerful hydroxyl-groups in the form of a peroxide. The adsorption is more marked in fixed specimens, as electrolytes play a more important *rôle*, but nevertheless oxidation and reduction come again to the fore, since reduction-sensitive stains are converted into their leuco-bases by those lipoid-globulin particles which are richer in active hydrogen than active oxygen, and *vice versâ*.

CHEMOTHERAPY.

Adsorption between the lipoid-protein particles of the resisting substance and emulsion colloids. Metallic compounds increase the amount of active oxygen directly by forming hydroxide-protein adsorption compounds, and non-metallic compounds increase the amount of active oxygen indirectly by forming di-sulphide-protein adsorption compounds.

Action is more marked in protozoal diseases, owing to the increased size and adsorptive capacity of lipoid-protein particles of the resisting substance in these diseases, but a continued alternate administration of the metallic and non-metallic compounds, which increase the natural ways of producing active oxygen and active hydrogen, benefit almost any chronic infection.

Change produced is an alteration of the permeability, and a breaking up of the bodies adsorbed.

Therefore the *rationale* of immunity, anaphylaxis, staining, and chemotherapy is fundamentally the same.

I trust that I have now explained how each link in my chain of reasoning was forged. I venture to think that each successive step follows logically from its predecessor. This is a strong argument in favour of the correctness of my deductions. In connection with these deductions, I would lay particular stress on the fact that I have not been compelled to evolve new terms for the sake of explaining my theories.

To be forced to evolve new terms is to be forced to confess that existing methods of logic are inadequate for purposes of explanation—in other words, to be forced to confess that the matter to be explained is incapable of logical explanation.

My theories may be wrong, but, at least, they follow simply and logically from ascertained facts, and I have deduced them entirely from established chemical and physical facts.

Bacon, the father of scientific investigation, said: "The true kind of experience is not the mere groping of a man in the dark, who feels at random to find his way, instead of waiting for the dawn or striking a light. It begins with an ordered—not chaotic—arrangement of facts, deduces axioms from these, and from the axioms again designs new experiments." I have endeavoured to follow the great advice expressed in these noble words.

I have laid my theories before my hearers, in the hope that they may receive the strictest scrutiny, and that this scrutiny may lead to further advances in the science to whose furtherance we all are pledged.

Finally, speaking solely in the interests of British medicine, in view of the facts that the *Spirochœta pallida* is not the sole cause of syphilis, that the Wassermann reaction and salvarsan and its arsenical substitutes will, I trust, soon be no longer necessary, I think I may say that the palm I referred to in opening these lectures has at last come to this country. I cannot close my lectures without thanking those who have given me so much assistance in my research work. I wish to thank particularly Mr. Ernest Lane, Dr. R. L. Mackenzie Wallis, Mr. J. Patterson, Dr. H. Spence, the British Drug Houses, Ltd., and Professor W. M. Bayliss, whose book "Principles of General Physiology" has been a great source of inspiration to me.

INDEX.

Abderhalden's test, 37, 38, 71.
Acetic acid, action of, 17.
—— —— ammonium sulphate test, 80.
—— —— lanthanum sulphate test, 84.
—— —— thorium nitrate test, 86.
—— —— uranium nitrate test, 87.
Acid receptors, fatty, 105.
Adsorption, 42, 52, 108, 189, 196.
—— active oxygen and, 107.
—— by syphilitic serum, 133.
—— compounds, 33.
—— factors in, 66.
—— *in vitro*, 191, 196.
—— *in vivo*, 191, 196.
—— non-specific, 197.
—— of dyes, 13.
—— of homologous molecules, 37.
—— reaction, 34.
—— specific, 69, 196.
Adsorptive capacity, 118.
Agglutination, 176.
Agglutinins, 26, 53.
Alexines, 25, 26.
Alexocytes, 25.
Alkalinity, production of, 124, 181.
Allergy, 91.
Aluminium, action of, 145.
—— hydroxide, 92, 146, 173.
—— mordanting, 20.
Aluvine, 132.
—— action of, 149.
—— formula of, 146.
—— non-toxicity of, 150.
Amboceptors, 25, 26.
Amœba, influence of hydrogen ion concentration on, 181.
Amino-groups, 37, 44.
—— action of, 40.
—— fixing of, 116.
—— specific, 66, 183.
Ammonium sulphate test, 80.
Ampholyte, 166.
Anæsthetics, effect in anaphylaxis, 103.
Anaphylaxis, 91, 193, 197.
—— anti-, 99.
—— disorders associated with, 95, 97.
Anaphylaxis, produced by proteins, 100.
—— symptoms of, 174.
—— *See also* Shock.
Animals, cold and warm blooded, immunity examples, 63.
Anions, effect of, 14.
Antibodies, 25, 32, 40.
—— action of, 99.
—— adsorption of, 42.
—— difference between antigens and, 70.
—— diagnosis by finding, 3.
Antigens, 40.
—— action of, 99.
—— adsorption of, 42.
—— difference between antibodies and, 70.
—— factor in Wassermann reaction, 31, 72.
—— gonococcal, 55.
Arsacetin, action of, 127.
Arsenic, action of, 105, 115, 125, 194.
—— compounds, chemistry of, 120.
—— —— earlier, 127.
—— —— French, 129.
—— preparations, anaphylaxis following use of, 102.
—— receptors, 104.
Arsenic-fast organisms, 106.
Arseno-phenyl-glycine, 128.
Asphyxia after intravenous injections, 95.
Asthma, anaphylactic, 97.
Atoxyl, action of, 127.

Bacteriolysis, 27.
Bacteriotropins, 26.
Barium sulphate, 33.
Bicarbonate system, 170.
Blepharoplasts, 10.
Blood corpuscles, hæmolysed, 34.
—— —— red, 108.
Blood-pressure, effect of anaphylaxis on, 95, 99.
Blues, aqueous solutions of, 15.
Borax-methylene blue, 15.

Calcium chloride, and anaphylactic shock, 93.

Calcium chloride, injections of, 98, 103.
—— ions, effect on phagocytosis, 180.
Carboxyl-groups, 43, 116.
—— —— *rôle* played by, 56.
Catalysts, 49.
—— action of, 115, 119, 178.
Cell membrane, 56, 58.
Cells, fertilisation of, 9.
—— staining of, 15.
Cerebro-spinal fluid in syphilis, content of, 112.
Chancres, induration in, 149.
Chemoceptors, 104.
Chemotherapy, 104, 198.
—— analogy of immunity to, 133.
—— rationale of, 4.
Child, syphilitic, from healthy mother, 4.
Chilblains, 161.
Chromatin, 17.
Clinical features of syphilis, 2.
Colloidal iodine, Crookes', 158.
—— nature of dyes, 12.
—— particles, 35, 192.
—— reaction, 33.
—— solutions, 40, 169.
—— —— surface tension of, 38.
Colloids, adsorption by syphilitic serum, 133.
—— anaphylactic shock and, 92.
—— and immunity, 40.
—— emulsion, 47.
—— —— activity of, 134.
—— intravenous injection of, 95.
—— suspension, 93.
Complement, anaphylactic changes in, 97.
—— destruction of, 45, 49, 72, 176.
—— division by dialysis, 28.
—— factor in Wassermann reaction, 34.
—— fixation tests, 3, 28, 54, 60.
—— —— —— non-specific, 31, 60.
—— middle and end pieces of, 50.
—— nature of, 24, 174.
—— precipitation of, 38.
Congenital syphilis, 159.
Congo red, 13.
Crookes' colloidal iodine, 158.
Cuti-reaction in syphilis, 101.

Dermatitis, anaphylactic, 95, 97.
Di-ortho-amino-thio-benzene. *See* Intramine.
Di-oxy-di-amino-stibino-benzene, 131.
Di-para-amino-manganese-benzene sulphonate, 144.
Di-para-amino-thio-benzene, 136.
Drugs, antisyphilitic, mode of action, 106.
—— new, clinical value of, 149.
—— —— synthetic anti-syphilitic, 135.
Ducrey's bacillus, 2.
Dyeing, 12, 191.
Dyes, action on proteins, 15.
—— adsorption of, 13.
—— basic, 16.
—— colloidal nature of, 12.
—— electro-negative, 14.
—— filtration of, 12.
—— fixation of, 14.
—— negative, 13.
—— positive, 12.
Dyestuffs, blending of, 121.

Ehrlich's discovery of salvarsan, 2.
—— theory of, 24.
—— views on salvarsan, 104.
Electrical dissociation, 175.
Electrolytes, action of, 52, 58.
—— amphoteric, 168.
—— effect of, 14.
—— importance of, in staining, 14.
—— insoluble, 166.
—— suspension colloids sensitive to, 93.
Emulsion colloids, activity of, 134.
Emulsoid-suspensoid test, 75.
Emulsoids, 54, 69, 169, 185.
Encephalitis, degenerative, 149, 154.
Enzymes, reduction by, 50.
—— Schardinger's, 171.
Eosin, action of, 15.
Eosin-methylene-blue mixture, 13.
Eosinophile cells, 97, 109.
Experimental inoculation, 1.

Fatty acids, influence on Wassermann reaction, 48.
—— —— unsaturated, 113.
Ferments, action of, 27, 111.
Ferric hydroxide, 33.
Ferrivine, 132.
—— action of, 149.
—— formula of, 146.
—— non-toxicity of, 150.
Fertilisation of cells, 8.
Filtration of dyes, 12.
Fixation of dyes, 14.
Formalin, action on amino-groups, 44.
Freezing point of sera, 33.

Galyl, 159.
—— formula of, 129.
—— intravenous injections of, 82.
Gamete, 10.

Gamete, male, 113.
Gametocyte, 10.
—— female, 8, 10.
—— male, 8, 10.
Generalisation stage, treatment of, 158.
Gibbs-Thomson law, 189.
Gold sol test, 53.
Gonococcal antigens, 55.
Gonorrhœa, inoculation experiments, 1.
Granules in epithelial cells, reactions of, 112.
Grey oil, intramuscular injections of, 157.

Hæmoglobin, 38.
Hæmolysis, 27.
—— cause of, 55, 57.
—— due to soaps, 27.
Hæmolytic system in Wassermann reaction, 34.
Heart, effect of intravenous injections on, 95.
Histological preparations, 14.
History of syphilis, 1.
Host, physical state of, 65.
Hunter (J.), inoculation experiments by, 1.
Hydrochloride, salvarsan, 123.
Hydrogen, active, 21.
—— ion concentration, 91, 171, 182, 192.
—— —— —— influence on amœba movements, 181.
—— positions, 109.
—— significance of, in biology, 165, 184.
Hydrogen-group, 50.
Hydrolysis, 38, 71.
Hydroxides, 47, 177.
—— injections of, 138.
Hydroxyl-groups, 50, 125.
Hydroxyl ion concentration, 66, 178, 183.
Hydroxyl ions, significance of, in biology, 165, 171, 184.
Hypersensitiveness, 91.

Immunity, acquired, 63, 65, 182.
—— action *in vivo* and *in vitro*, 196, 197.
—— analogy to chemotherapy, 133.
—— —— of to staining, 62.
—— and colloids, 40.
—— arsenical, 117.
—— artificial, 188.
—— in animals, 63.
—— main theories of, 24.
Immunity, natural, 65, 182, 188.
—— pyronin, 117.
—— reactions, individual, 60.
Infections, production of, 65.
Injections, anaphylactic shock after, 95.
—— intravenous, advantages of, 146.
Inoculation experiments by Hunter, 1.
Insolubility, 166.
Intramine, 132.
—— formula of, 136.
—— injection of, 149.
—— intramuscular injections of, 82.
—— iodides increase action of, 157.
—— non-toxicity of, 150.
—— mode of action of, 138.
—— pain after injections of, 160.
—— preparation of, 136.
—— solution *in corpore*, 168.
Intramine-lecithin, 137.
Intravenous injections, anaphylactic shock after, 95.
Iodides, action of intramine increased by, 157.
—— following treatment by salvarsan, 154.
Iodine, action of, 142, 195.
—— Crookes' colloidal, 158.
Ions, balance between, 171.
—— dissociation of, 45, 175.
—— hydrogen, 91, 171, 175.
—— hydroxyl, 165, 175.
—— uni-valent, 54.
Iron, action of, 147, 195.
—— hydroxide, 177.

Kaolin, 33.
Kations, effect of, 14.
Keratitis, interstitial, 149.
Kharsivan, 159.
Kidney, effect of intravenous injections on, 96.

Lange's test, 54.
Lanthanum sulphate test, 84, 88.
Lecithin, intramine put up with, 137.
Lecithin-globulin, 17.
Leprosy, intramine in, 161.
Leucocytes, alexines from, 25.
Leucocytozoon syphilidis, 7, 9, 187.
—— —— phases of, 51.
—— —— staining of, 18, 59.
Life-cycle of organism of syphilis, 7.
"Lipoid," 56.
Lipoid-globulin, 69.
—— complex, 34.
—— molecules, 10, 35, 41.
—— —— actions of, 108.
—— —— anti-protozoal, 107.

Lipoid-globulin molecules, breaking down of, 118.
—— —— in serum, 108.
—— particles, 43, 46, 73.
—— —— in serum, 166, 170.
Lipoid-protein, 42, 69.
—— —— molecules, 19.
—— —— particles, 52.
Lipoid solvents, 57.
Liver, fœtal, syphilitic, 3.
Ludyl, 129.
Lupus treated with intramine, 152.
Lymphatic glands, examination of, 5.

Malachite green, 17.
Malignant disease, etiology of, 36.
—— —— intramine in, 161.
Manganese, 143.
Margol, 122.
Mast cells, increase of, 97, 109.
Meningeal syphilis, 153.
Meningitis, cerebro-spinal, 149, 156.
Meningo-encephalitis, 159.
Mercaptan-protein, 140, 178.
Mercury, following treatment by salvarsan, 154.
—— mechanical action of, 153, 195.
Merozoites, male and female, 7, 10.
—— sexual, 7, 10.
Metallic compounds, 143, 194.
—— —— action of, 114.
Metals, inimical to the parasite, 179.
Methyl green stain, 17, 19, 21.
Methylene blue, 17, 173.
—— red, 15, 17, 173.
—— violet, 16, 17, 19, 173.
Molecules, adsorptive capacity of, 119.
—— homologous, adsorption of, 37.
Mordants, 20, 174.
Myelitis, degenerative, 154.
—— —— treatment of, 159.

Negative reaction, 4.
Neo-kharsivan, 121, 159.
Neo-salvarsan, composition of, 125.
—— substitution products for, 121.
Ninhydrin reaction, positive, 37.
Non-metallic compounds, 135.
Nuclei, extracts of, 26.
—— staining of, 19, 21, 22.

Opsonins, 26.
Oxidase-reducase system, 49, 65, 183.
—— —— action of intramine in, 139.
Oxidase system, 20, 171.
Oxidases, 51.
Oxidation, 45, 108.
—— and reduction, 19.
—— process, 171, 178.
Oxidation, staining by, 18.
Oxidising remedies, continual administration of, 153.
Oxygen, active, 50, 69, 109, 171, 190.
—— —— adsorption and, 107.
—— anaphylaxis, 102.
—— "chain," 108.
—— positions, 110.
—— relationship to sulphur, 140.

Pain, after injections, 160.
—— after intramuscular injections, 136.
Papules, syphilitic, 149.
Pappenheim's stain, 18.
Parasites, metals inimical to, 179.
—— salvarsan-fast, 117.
Parent, healthy, with syphilitic child, 4.
Particles, size of, 40.
Perhydridase, 110, 171.
Peroxidases, 19, 46, 108, 171.
—— artificial, 47.
Peroxides, 19, 108.
Phagocytosis, 26, 180.
Philothion, 138.
Phosphate system, 170.
Phosphorus, 132.
Pigment, 145.
Piles, 162.
Placental extract, 36.
Plasma cells, specificity of, 106.
Plasmomata, 107.
Polar bodies, expulsion of, 9.
Polychrome methylene blue, 16.
Positive reaction, 3.
Precipitation, 38, 176.
Precipitins, 26, 53.
Pregnant woman, action of serum of, 36, 37.
Primary stage, treatment of, 157.
Proteins, action of dyes on, 15.
—— adsorption of, 42.
—— anaphylaxis produced by, 92, 94, 101.
—— mercaptan, 140.
—— properties of, 5.
—— serum, physical and chemical properties, 77.
Protein-precipitants, 33.
Protoplasm, as base or acid, 18.
Protozoal diseases, serum of, 107.
—— Wassermann reaction positive in, 40.
Protozoon, life history of, 5.
Pyronin, 17, 19, 21.
—— formula of, 117.
—— staining with, 19.

Radium, action of, 161.
Reactions, individual immunity, 60.
—— significance of, 4.
—— *See also* Wassermann reaction.
Reagin, 32.
—— action of, 37.
—— effect of temperature on, 32.
—— particles, 43.
Receptors, 25, 104.
—— Ehrlich's view of, 105.
Recurrence, stage of, treatment of, 154.
Reducase system, 20.
Reducases, 51.
Reducing remedies, continued administration of, 153, 155.
—— substances, 138.
Reduction, 45, 108.
—— and oxidation, 19.
—— process, 177.
—— stains, 17.
Resisting substance, action of, 27.
Ricord, unicity of the two diseases disproved by, 1.
Rinderpest, 64.
Rongalit white, 13, 17.

Saline, hypertonic, injections of, 98.
Salts, free, increase by freezing, 33.
—— neutral, 13.
Salvarsan, action of, 194.
—— anaphylaxis after three injections, 101.
—— changes in cells due to, 40.
—— composition of, 123.
—— continued injections of, 4.
—— discovery of, 2, 105.
—— French substitutes for, 159.
—— not fatal to parasites, 117.
—— recurrences after use of, 154.
—— substitution products for, 121.
Schardinger's enzyme, 171.
Schaudinn's discovery of *Spirochæta pallida*, 2.
Schizogony, 8.
Secondary syphilis, 157.
Sensitiveness of organs, 92.
Sera, freezing of, 33.
—— surface tension of, 39.
—— syphilitic, 35, 37.
—— —— positive ninhydrin reaction, 38.
Serum, acetic acid ammonium sulphate test applied to, 80.
—— complementoid, 63.
—— constituents of 68.
—— horse, action of, 99.
—— inactivated, 48.
—— ions in, 171.
Serum, lipoid-globulin molecules in, 108.
—— —— particles in, 166, 168.
—— normal, acetic acid lanthanum sulphate test with, 84, 88.
—— —— acetic acid thorium nitrate test with, 86, 88.
—— —— acetic acid uranium nitrate test, 87, 88.
—— of pregnant woman, action of, 36.
—— protein particles in, 26.
—— proteins, physical and chemical properties, 77.
—— of protozoal diseases, 107.
—— syphilitic, acetic acid lanthanum sulphate test with, 84, 89.
—— —— acetic acid thorium nitrate test with, 86, 89.
—— —— acetic acid uranium nitrate test, 87, 89.
—— —— colloid adsorbed by, 133.
Sexual merozoites, 7.
Shock, anaphylactic, 92, 173.
—— —— prevention of, 103.
"Side-chain" theory, 24.
Silver nitrate, staining with, 114.
Soaps, hæmolysis due to, 27.
Sodium chloride, injections of, 98.
—— —— precipitating action, 54.
Soft sore, 2.
—— —— chronic, 162.
Sols, colour of, 53.
—— positive and negative, 173.
Solubility, 166, 185.
Solutions, colloidal, 40.
—— colloidal and true, 169.
Specific reaction, 36.
Specificity, 34, 36, 40.
Spermatozoon, 5.
Spirochæta pallida, 187.
—— —— destruction of, 113.
—— —— discovery of, 2.
—— —— division of, 4.
—— —— extract of, 35.
—— —— staining of, 16, 58.
—— *refringens*, 8.
Spores, development of, 65, 183.
—— dormant, 155.
Sporoblasts, 9, 10.
Sporozoa, 9.
Sporozoites, 16.
—— division of, 7, 10.
—— staining of, 7.
Staining, 12, 14, 191, 197.
—— analogy of immunity to, 62.
—— importance of electrolytes in, 14.
—— *in vivo*, 16, 191.
Stains, Pappenheim's, 18.

Stains, pyronin, 19.
—— reduction, 17.
"Substance sensibilisatrice," 25.
Sulphate tests, 80, 84.
Sulphur, 132, 135, 195.
—— in reduction process, 138, 177.
—— relationship of oxygen to, 140.
Surface tension, 71, 169.
—— —— of colloidal solutions, 38.
—— —— of sera, 39, 48.
Suspensoids, 54, 69, 71, 169, 185.
Synthetic anti-syphilitic drugs, new, 135.
Syphilis, development of spore of, 66.
—— drugs in, mode of action of, 106.
—— early, metallic treatment of, 102, 138, 179.
—— in animals, 64.
—— in women, 41.
—— late, intramine treatment of, 102, 138, 141, 179.
—— test for, new and rational, 75.
—— visceral, 2.

Telosporidia, 9.
Temperature, effect on hydrogen ion concentration, 182.
—— effect on size of reagin-molecule, 32.
Test, acetic acid ammonium sulphate, 80.
—— for syphilis, a new and rational, 75.
Tests, technique common to all, 79.
Thorium nitrate test, 86, 88.
Treatment continued after disappearance of symptoms, 153.
Treatment, methods of, 149.
—— mixed, 157.
Trophozoite, 10.
Tropin, 26.
Trypanosomes, 106.

Ulcers, 162.
Ulcus molle, 2, 181.
Ulcus molle serpiginosum, 181.
Unicity of gonorrhœa and syphilis disproved, 1.
Uranium nitrate test, 87, 88.
Urticaria pigmentosa, anaphylactic, 97.

Valency, 131.
Vaso-dilatation, anaphylactic, 99.
Visceral syphilis, 2.

Wassermann reaction, 31, 39, 60.
—— —— antigen factor in, 32, 72.
—— —— complement factor in, 34.
—— —— discovery of, 2.
—— —— hæmolytic system in, 34.
—— —— influence of fatty acids on, 48.
—— —— marked in late syphilis, 35.
—— —— nature of, 5.
—— —— positive in protozoal diseases, 40.
—— —— principles underlying, 75.
—— —— rationale of, 10, 61.
Water in serum, 68.
Women, syphilis in, 41, 159.
Wounds, 162.

Zygote, 9, 10.

WELLCOME LIBRARY INSTITUTE

Harrison & Sons, Printers in Ordinary to His Majesty, St. Martin's Lane.